The complex foraging strategy of the specialised gall fly *Urophora cardui* (Diptera: Tephritidae) for host plants (*Cirsium arvense*, Asteraceae)

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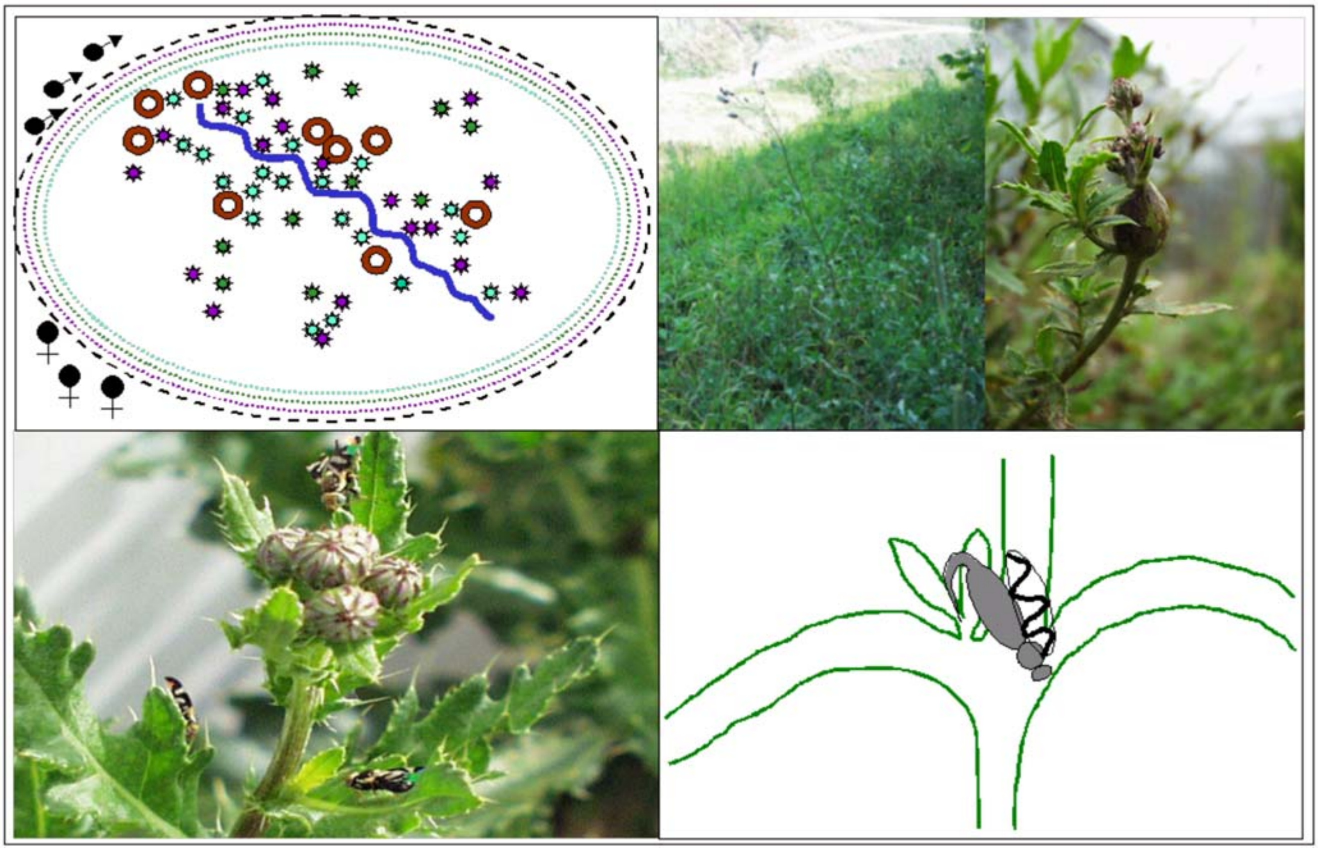

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Dissertation

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Vorgelegt von

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Damit das Mögliche entsteht, muss immer wieder das Unmögliche versucht werden.

Hermann Hesse

...meinen Eltern und meinem Mann gewidmet

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Erklärung zur Form der Arbeit und zum Eigenanteil an den einzelnen Kapiteln

For all international readers: please ignore the following part.

Bei der vorliegenden Arbeit handelt es sich um eine Arbeit im kumulativen Stil, auch wenn die bisherigen Manuskripte aufgrund zeitlicher Schwierigkeiten noch nicht bei Zeitschriften eingereicht werden konnten. Der Stil der Arbeit wurde an den Vorgaben der Promotionskommission der Universität Bayreuth orientiert. Dies bedeutet unter anderem, dass die für die Veröffentlichung geplanten Autoren mit Nennung des „corresponding authors“ angegeben sind, ferner die Angabe *aller* Methoden in *jedem* Kapitel, auch wenn dies zu Redundanzen führt, sowie die einzelne Auflistung der Referenzen nach jedem Kapitel, auch wenn dies zu Redundanzen führen sollte.

Wie für eine Arbeit im kumulativen Stil notwendig, wird im folgenden mein Eigenanteil an den jeweiligen Kapiteln dargestellt:

Das Thema wurde von Prof. Erwin Beck im Rahmen des Graduiertenkollegs 678 als Doktorarbeitsthema angeboten. Die Schwerpunktsetzung innerhalb des Themas, sowie die Versuchsplanung und –entwicklung wurden von mir selbständig durchgeführt.

- *Introduction*: hierbei handelt es sich um die geforderte allgemeine Einleitung, die sowohl zum Thema der Arbeit hinführen soll, als auch den Zusammenhang zwischen den einzelnen Unterfragestellungen des Themas zu verdeutlichen. Dieses Kapitel wurde von mir selbständig erstellt (Eigenanteil von 100%).
- *Life history of* Urophora cardui *(Diptera: Tephritidae):* sowohl der Literaturüberblick über die Biologie des Untersuchungssystems, als auch die vorgestellten Daten wurden von mir selbständig erhoben und ausgewertet (95% Eigenanteil). Prof. Helmut Zwölfer stellte mir einige unbekannte Literatur zur Verfügung, sowohl er, als auch Prof. Erwin Beck ließen einige Anmerkungen und Hinweise einfließen (beide zusammen etwa ein Beitrag von 5%).
- *Behavioural aspects of* Urophora cardui *(Diptera: Tephritidae) in relation to the host plant* Cirsium arvense *(Asteraceae):* die angegebenen Daten wurden von mir selbständig erhoben und ausgewertet, inklusive der Entwicklung des Versuchsdesigns (95% Eigenanteil). Sowohl Prof. Helmut Zwölfer, als auch Prof. Erwin Beck trugen einige Anmerkungen, Ideen und Hinweise bei (beide zusammen ein Anteil von ca. 5%).
- *The host plant recognition and the decision-time of* Urophora cardui *(Diptera: Tephritidae) in a stand of different plants:* das Versuchsdesign wurde von mir entwickelt, die Daten selbständig erhoben und ausgewertet (95% Eigenanteil). Prof. Helmut Zwölfer und Prof. Erwin Beck steuerten einige Anmerkungen, Hinweise und Ideen bei (beide zusammen ein Anteil von ca. 5%).
- *How do males and females of the specialized gall fly* Urophora cardui *(Diptera: Tephritidae) select for a particular suitable host plant?:* das Versuchsdesign

wurde von mir entwickelt, die Daten selbständig erhoben und ausgewertet (90% Eigenanteil). Prof. Helmut Zwölfer und Prof. Erwin Beck steuerten einige Anmerkungen, Hinweise und Ideen bei (beide zusammen ein Anteil von ca. 5%). Prof Konrad Fiedler lieferte einige wertvolle Hinweise zur statistischen Auswertung der Versuche (Anteil von ca. 5%). Herr Stefan Dötterl erlaubte mir die Benutzung des für ihn von der Werkstatt gebauten Windkanals.

- *Morphology of the ovipositor of the thistle-stem gall fly* Urophora cardui *(Diptera: Tephritidae):* die Daten wurden von mir selbständig erhoben und ausgewertet (ca. 95% Eigenanteil). Prof. Georg Acker und Prof. Helmut Zwölfer (beide zusammen ein Anteil von ca. etwa 5%) lieferten einige Tips und Anmerkungen für die Auswertung, Prof. Helmut Zwölfer stellte außerdem Literatur bereit, deren Beschaffung schwierig war. Da die Elektronenmikroskopie für ihre Dienste bezahlt wurde, findet Prof. Georg Acker keine Aufnahme als Autor des Manuskriptes. Prof. Erwin Beck, als mein Doktorvater wird jedoch auf der geplanten Veröffentlichung genannt sein.
- *The selection of a suitable axillary bud for oviposition by* Urophora cardui *females (Diptera: Tephritidae):* das Versuchsdesign wurde von mir entwickelt, die Versuche selbständig durchgeführt und ausgewertet (ca. 90% Eigenanteil). Prof. Konrad Fiedler gab einige Hinweise zur möglichen statistischen Auswertung der Versuche und ermöglichte die Benutzung des Statistikprogrammes „Statistica“, das am Lehrstuhl für Pflanzenphysiologie nicht zur Verfügung stand, sich für die Auswertung jedoch als notwendig herausstellte (Anteil von ca. 5%). Prof. Helmut Zwölfer und Prof. Erwin Beck gaben einige Hinweise, Ideen und Anmerkungen (beide zusammen ein Anteil von ca. 5%).
- *Résumé: Model of the foraging for oviposition sites by* Urophora cardui*:* diese Zusammenfassung der Ergebnisse aller Kapitel wurde von mir selbständig erstellt (100% Eigenanteil).
- *Synopsis:* bei diesem Abschnitt handelt es sich um die für eine kumulative Arbeit geforderte Zusammenschau der verschiedenen Kapitel, in der die Verbindung zwischen den einzelnen Arbeiten dargestellt und zusammenhängend diskutiert wird. Dieses Kapitel wurde von mir vollständig selbständig verfasst (100% Eigenanteil).

Kleinere Hilfsleitungen von Einzelpersonen, wie beispielsweise Korrekturlesungen, sowie die finanzielle Förderung sind in den jeweiligen Danksagungen am Kapitelende aufgeführt. Während der gesamten Arbeit wurden von mir keine Praktikanten oder Diplomanden betreut. Somit werden keine Daten anderer Personen veröffentlicht.

Abbreviations

CEST	Central European summertime
df	degrees of freedom
Fig.	Figure
K-S	Kolmogoroff Smirnoff test of normal distribution
L1	first larval instar
L2	second larval instar
L3	third larval instar
L:D	light: dark period
Magn.	Magnification
Mann-Whitney-test	Mann-Whitney U-test
n	sample size
pers. comm.	personal communication
R_S	correlation coefficient of a Spearman-Rank correlation
S.D.	standard deviation, generally noted after the mean as ± x
SEM	scanning electron microscopy
TEM	transmission electron microscopy
Wilcoxon test	Wilcoxon signed rank test
χ^2	chi-square
ø	diameter

1 Introduction

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The great majority of all animals on earth interact with plants in their effort to survive and reproduce (Schoonhoven, 1990). For herbivorous insects, interactions with plants are of critical importance, since plants are not only used as a food source but also as a place for mating and egg-deposition (Feeny, 1975; Price *et al.*, 1980; Schoonhoven, 1990; Schoonhoven & Jermy 1998). In turn, plants are subject to selection forced upon them through usage by the insects (Schoonhoven & Jermy, 1998). Other influences, for example human usage, climate, fire or grazing by macro-herbivores also pose selection-pressures on the first trophic level, but herbivorous insects remain the most abundant group, measured either by species abundance or individual abundance (Schoonhoven, 1990).

Since they set the initial conditions for nearly all the following processes in the microhabitat, mating and oviposition are the key behavioural patterns performed by herbivorous insects (Dettner *et al.,* 1997b; Price, 1992; Schoonhoven & Jermy, 1998; Strong *et al.,* 1984; Zwölfer, 1994). Mating is not only a prerequisite of the reproductive success of the individuals involved, it also has the consequence of facilitating evolution (Headrick & Goeden, 1994; Jiggins & Bridle, 2004). In many herbivorous insects oviposition is closely linked to mating (Papaj, 1994; Raghu *et al.*, 2002). Both behaviours also play an important role on population dynamics (Preszler & Price, 1988; Price *et al.*, 1990). If the functions of mating and oviposition of herbivorous insects in terrestrial ecosystems are considered in this ecological background they also have an influence on population dynamics of other species (Masters & Brown, 1997).

For a better comprehension of the biology of one species, including its population structure, it is necessary to conduct a careful analysis of all interactions with other species (Cornell & Lawton, 1992; Tscharntke, 1992). Such a food web analysis helps unravel evolutionary processes (Dettner *et al.*, 1997; Price, 1992; Zwölfer & Arnold-Rinehart, 1993). Furthermore, such analyses provide a useful tool in the search for general patterns of population dynamics (Price *et al.*, 1995). With the growing concern for the environment in the last years the study of food webs has become also politically important (Harris, 1991).

Investigation of mating and oviposition combines several major theoretical areas in evolutionary ecology and is thus an exciting and rewarding area of research (Charnov & Skinner, 1985): these include game theory (Parker & Smith, 1990), life history theory (Fritz *et al.*, 2003) and foraging theory (Pyke, 1984). Recently chemical ecology has become important in regard to mating and oviposition, too (Dutton *et al.*, 2000). Behavioural plasticity is also of importance (Casiraghi *et al.*, 2001) as are the morphology of the ovipositor and the antennae (Vilhelmsen *et al.*, 2001). On the one hand structural constraints of both organs limit the host plant range of the insect (Fletcher & Prokopy, 1991), although on the other hand the morphology and sensitivity of the receptors on the ovipositor and the antennae are improved by adaptive selection, which enhances reproductive fitness by improving the host plant recognition ability of the insect (Altner & Prillinger, 1980; Brown & Anderson, 1998).

The complex behaviour of host plant selection by herbivorous insects will now be described in more detail. In the course of host plant choice, the quality of the plant should be crucial for the female (Burkhardt & Zwölfer, 2002; Hassell & Southwood, 1978; Mappes & Kaitala, 1995), since higher quality ensures a higher larval survival (Baylis & Pierce, 1991). Often host plant quality is associated with the nitrogen supply of the plant (Fox *et al.*, 1990; Stamp & Bowers, 1990), but other aspects, like water supply can also affect quality for a certain insect species (Horner & Abrahamson, 1992; Preszler & Price, 1988; Ramløv & Lee, 2000; Waring & Price, 1988). High host plant quality does not necessarily imply that the host plant performs well (de Bruyn, 1995; Scheirs & de Bruyn, 2002). Not only host quality directly, but more basically the quality of the host's habitat is important, since it may influence host density and additionally host quality (Diaz-Fleischer & Aluja, 2003). This interface between the host plant and its abiotic growing conditions can act as an evolutionary force on the insect's dispersal strategies (Holt & Lawton, 1993; Simberloff & Stiling, 1996), host plant selection strategies and population density (Price, 1992). Thus the effects of the abiotic environment via the larval host play a central role for the herbivorous insect in determining time and space of population densities and performance (Price, 1992).

A further feature, which often has a significant impact on herbivorous insects, is the influence of natural enemies, respectively predators and parasitoids, the latter being often more important (Bernays, 1990). For an analysis of the selection for a suitable host plant in herbivorous insects it is not only important to include the third or the first trophic level, but to take also interactions between all three levels into account (Achtziger, 1997; Micha *et al.*, 2000; Shiojiri & Takabayashi, 2003). Plant density and the size of plant stands will affect the herbivore and its predators and parasitoids alike (Price *et al.*, 1980). Plant density has also indirect effects on the performance of the larvae of a particular herbivore and its associated parasitoid and predator species, since plant density influences the microclimate (Feeny, 1970; Price, 1988), nutritional quality (Scriber & Slansky, 1981; Stamp & Bowers, 1990), the concentration of certain nutrients in the plant tissue, and the presence of secondary plant volatiles (Cornell, 1983; Evans, 1990; Monaco *et al.*, 1982; Schoonhoven, 1972). The latter are considered to attract parasitoids especially in order to control the herbivore (Bouletreau, 1986; Dutton *et al.*, 2000). However secondary plant volatiles also can be used as signals by the herbivorous insect (Bäckmann *et al.*, 2001; Ignacimuthu *et al.*, 2000; Müller & Hilker, 2000; Turlings *et al.*, 1990).

Several years ago it was quite popular to discuss the role of top-down (exerted by parasitoids or predators) and bottom-up (generated by resource availability) effects in terrestrial ecosystems (for example Power, 1992; Scheirs & de Bruyn, 2002) and the topic is still controversial. In complex systems with at least three trophic levels, it is difficult to assess the influence of one of these forces. The presence and quality of host plants is often as important (for example Baylis & Pierce, 1991; Obermaier & Zwölfer, 1999; Stamp & Bowers, 1990) as protection from natural enemies (for example Ballabeni *et al.*, 2001; Berdegue *et al.*, 1996; Lill *et al.*, 2002) or density-dependent dispersal of the phytophagous insect (for example Dempster *et al.*, 1995; Freese, 1995; Zwölfer & Völkl, 1997). These effects may interact with each other (Biere *et al.*, 2002), they may change each others importance or be similarly important (Hunter & Price, 1992).

Because these complex systems are difficult to study, smaller models such as self-contained small eco-systems like plant galls (Angermann, 1986) of herbivorous insects (Mani, 1964) are used: these are where the larvae develop (Bronner, 1992; Mani, 1992). Since the larvae are not able to leave their host plant, accurate host plant selection by the female becomes crucial (Ballabeni *et al.*, 2001), since the larvae are not able to compensate for a non-adaptive choice performed by their mother. In this case the behaviour of the female sets the initial conditions from which any larval interaction may proceed (Mayhew, 1997). The evolution of these gall structures is described as an example of unilateral exploitation of the morphogenetic capacities of the plant, since in this case it is not beneficial for the plant (Allee *et al.,* 1967). This is one of the reasons, why gall insects are often used in biological control of weeds (Harris, 1989; Harris & Shorthouse, 1996; Peschken *et al.*, 1982; Redfern & Cameron, 1989; Zwölfer, 1968).

Nowadays studies that can be related to biological control (Harris, 1991) of weed or pest species have become important once more (Baars *et al.*, 2003; Denoth *et al.*, 2002; Jiggins & Bridle, 2004; Kruess, 2002). After the first enthusiasm about having found a non-chemical, cheap and highly successful method of weed and pest control, several critical arguments about general practice in biological control have been raised (Baars *et al.*, 2003; Denoth *et al.*, 2002). It therefore becomes necessary to study the causes of indirect effects, in order to avoid deleterious effects in future release projects and to understand their mechanisms. If host-specific biocontrol agents establish in a new habitat, but are not able to reduce their hosts' density, indirect effects can link the target weed to other native organisms and thereby expand the impacts of the invasive weed (Pearson & Callaway, 2003). Today we have a worldwide situation of introduced exotic species, acting as biocontrol agents, outnumbering exotic weed species for which they were introduced (Denoth *et al.*, 2002). Extensive studies of the host plant selection behaviour of galling insects before their integration in control projects is therefore of a critical importance (Harris, 1989; Harris & Zwölfer, 1968; Harris, 1991)

Host plant selection is one of the most complex behavioural sequences in insects. It involves different defined steps, which have to follow each other in a sequence. In the literature many different classifications of these steps can be found, depending on the study system (Fletcher & Prokopy, 1991; Mangel & Roitberg, 1993; Mangel & Roitberg, 1989; Nagelkerke, 1994; Rosenheim & Mangel, 1994). The number of these steps also vary according to the study system (Mangel & Roitberg, 1989; Roitberg, 1990; Rosenheim & Mangel, 1994). From step to step, detection and selection of a suitable larval host become more finely tuned (Zwölfer, 1968).

The first task of the female is to select a certain habitat and a special searching strategy is needed in order to detect host plant stands (Nordlund, 1994; Weis & Kapelinski, 1984; Whitham, 1978). The searching strategy includes features of time allocation to a single host plant stand; it determines how many patches a female will try to visit and how many eggs are deposited in each stand (Nagelkerke, 1994; Roitberg, 1990). Once in a host plant stand, the female has to select the plant that seems most suitable for larval development (Bernays & Chapman 1994; Mayhew, 1997; West & Cunningham, 2002). After arriving on this selected plant, the female still has to find a particular plant organ that is suitable for oviposition (Chun & Schoonhoven, 1973; Dutton *et al.*, 2000; Hattori, 1988; Lynn & Chang, 1990). Afterwards, the clutch size has to be adjusted to the host plant quality (Godfray, 1987; Lalonde &

Roitberg, 1994). In Hymenoptera the female additionally faces the question of the sex ratio to produce (Craig *et al.*, 1992; Fox *et al.*, 1990).

In the course of the present study host plant recognition and selection was studied in the specialised gall fly *Urophora cardui* on its host plant *Cirsium arvense. U. cardui* is one of the two only *Urophora* species, which induces galls not in the thistle head but in the stem (the other species is *U. misakiana* on *C. setidens* in East Asia (H. Zwölfer pers. comm.)). Nearly all sister taxa occur on Carduae. Gall formation is obviously a derived character (Zwölfer & Arnold-Rinehart, 1993). The ancestor was presumably achene mining (Zwölfer & Rinehart, 1994; Zwölfer & Arnold-Rinehart, 1993). The gall, which is induced by *U. cardui,* is the most derived in the genus. Gall structure enables the larvae to recruit nutrients from the whole host plant, not only the galled side shoot (Lalonde & Shorthouse, 1985). Thus the study species is of evolutionary interest. Additionally the larvae are highly dependent on host plant fitness, which implies that an optimal choice by the females is necessary, which makes *U. cardui* a suitable object for the study of foraging strategies.

C. arvense is an aggressive weed species, not only in Europe, but also in the northern USA and Canada, where it has been accidentally introduced. *U. cardui,* as one of its specialised herbivores, was established as a biocontrol agent in the 1970s (Peschken & Harris, 1975). The release of *U. cardui* as a biological control agent provides preliminary information about its host plant selection process, especially in relation to habitat characteristics (Peschken & Derby, 1997; Peschken *et al.*, 1982; Peschken & Harris, 1975; Shorthouse & Lalonde, 1986): it became obvious that host plant usage is low compared to other species (Goeden, 1987), which is presumably related to its complex and rigid selection behaviour.

After a description of important life history factors and the biology of the study system the following nine questions are posed:

1) What are the main characteristics of foraging behaviour in *U. cardui*?
2) How rigid is the host plant template of *U. cardui* exhibited in different choice situations?
3) How do males and females of *U. cardui* select for a suitable host plant stand?
4) Which factors determine the time until a decision for a particular host plant is derived?
5) Which host plant cues are important for the selection by females of *U. cardui*?
6) Which senses are used by males and females for host plant recognition and selection?
7) Is there any impact of the substance the males use for territory marking?
8) What is the ultrastructure of the ovipositor like, which receptors can be found on it and what implications can be derived for the selection process of a particular host plant organ?
9) Which host plant cues are used by the females to select a certain host plant organ as oviposition site?

After the particular chapters, which cover these different questions, a model of the host plant selection process by *U. cardui* is discussed. This is followed by the synopsis.

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2 Life history of *Urophora cardui* (Diptera: Tephritidae)

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Females of the specialised gall fly *Urophora cardui* oviposit into axillary buds of the creeping thistle *Cirsium arvense*. The fly larvae hibernate inside the gall. Life history characteristics as body size, longevity and larval development of herbivorous insects influence their foraging for larval host plants. As a background for a study on female search for oviposition places in this gall system a review of the existing literature was performed. Additionally some general parameters of the study system were analysed.

It was demonstrated that females of *U. cardui* are heavier than the males. In contrast the head capsule width of both sexes is similar. It is remarkable that the different measures of body size analysed here, weight at eclosure, death weight and head capsule width are only marginally correlated with each other. There is a significant difference between death weight and the weight of the animals at eclosure. The longevity of both sexes is not correlated with their body size measured as the weight at eclosure.

Neither the presence of several days at low temperature (cold storage simulating winter days), nor the duration of such a cold period had an influence on the emergence rate. Therefore in the following experiments, data from flies that had encountered different durations of cold period during larval development could be pooled.

2.1 Introduction

Life history traits are not only the basis of many further aspects of animal ecology (Fritz *et al.*, 2003; Price, 1992b; Stearns, 1992), and thus important as a background information of all ecological studies, but they themselves are important for a better understanding of the biology of the studied species (Leather, 1990; Palmer & Strathmann, 1981). Insect-insect interactions, insect-plant interactions and interactions with environmental factors are influenced by the life history traits of the respective species (Charnov & Skinner, 1988). Host plant choice and sexual selection are especially influenced by life history traits of herbivorous insects (Nylin & Gotthard, 1998), since these aspects are the first to be influenced by evolution.

2.1.1 Life history theory

Life history theory in general is the theory of life cycles, particularly in regard to fitness (Nylin & Gotthard, 1998). In their review on life history trait plasticity (Nylin & Gotthard, 1998) specify generation time and fecundity as the two most important factors. Both are influenced not only by the abiotic and biotic conditions in the habitat (Leimar & Norberg, 1997), but also by the resources available for an individual during its larval growth (Stearns, 1992), by behaviour (Roitberg *et al.*, 1982), especially foraging behaviour (including not only foraging for food, but also for oviposition sites and the search of mating partners), and also by interactions with conspecifics (Nylin & Gotthard, 1998). Regarding fecundity as a simple trade-off between female size and searching time for oviposition places would be too simple for the description of natural systems. Nylin & Gotthard (1998) expect a three dimensional framework between time, size and growth rate as the natural condition. These traits are influenced by each other as well as by other traits like behaviour, genomic information and interactions with conspecifics and the host plant. The predictability of suitable host plants in time and space and their quality are the main factors, which have an impact on the life history strategies of fruit flies (Tephritidae) (Fletcher, 1989). Predictability of host plants in time and space and their quality have thus mainly influenced the evolution of life history, the potential fecundity of females and the clutch size they produce (Fletcher, 1989). A female must be able to analyse the resource value of host plant(s) (units) prior to oviposition, which requires a finely tuned foraging strategy (Hassell & Southwood, 1978; Pyke, 1984). Foraging involves the usage of multi-sensory information and is generally time-consuming and thus costly (Roitberg, 1989). It is worth mentioning that among the specialised Tephritidae, which use flower buds as oviposition sites, there are also several pest species (Bush, 1969; Freidberg, 1984), even however these species, which are time limited as they produce usually extremely high clutch sizes in a very short time (Diaz-Fleischer & Aluja, 2003; Harris, 1989; Roitberg, 1989), require multi-sensory information (Diaz-Fleischer *et al.*, 1999).

While studying the life history of herbivorous insects from an evolutionary point of view, one can distinguish between adaptive syndromes and emergent properties (Price, 1994). Adaptive syndromes are the responses to phylogenetic constraints and thus describe optimisation of larval performance (Price, 1994). They also include larval adaptations to the oviposition decisions of females (for example adaptation to the selected host plant species). Emergent properties on the other hand are the consequences that result from the morphology, behaviour and physiology of the organisms in question (Price, 1994). In order to optimise oviposition behaviour, these emergent properties, especially behaviour and physiology, should be shaped by larval performance (Charnov & Skinner, 1988). The term “optimal oviposition behaviour” is widespread in literature (Charnov, 1976; Craig *et al.*, 1989; Parker & Smith, 1990; Pyke, 1984), but as Price (1992b) discusses, it is often difficult to access. It may be even more difficult to achieve in nature, although there is a high impact of larval performance on the selection of oviposition sites (Price, 1992a). The difficulties arise as a result of trade offs, for example between life span and reproduction (Smith & Fretwell, 1974; Weisser *et al.*, 1994). Enhanced egg production must be paid for in terms of reduced life span (Leather, 1990; Weisser *et al.*, 1994), reduced offspring size or reduced migratory activity (Leather, 1990). In this respect the quality of the larval host plant becomes increasingly important (Brewer & Skuhravý, 1980; Myers, 1985; Singer *et al.*, 1988; Williams *et al.*, 1997). In a variety of insect species the first offspring of a female usually has a higher reproductive fitness than all the following offspring (Leather, 1990), still emphasizing even more the importance of optimal host plant selection in the first attempt (Craig *et al.*, 1989). However, field studies often reveal sub-optimal egg deposition (H. Zwölfer pers. comm.). The possible reasons and processes, which lead to sub-optimal behaviour by females are poorly understood (Fritz *et al.*, 2000). However reproductive fitness may still depend on the quality of the host plant to a large degree. Fritz *et al.* (2003), for example, showed this in the case of *Phyllocolpa leavitti* (Hymenoptera: Tenthredinae). The growth rate of an individual depends on the plant quality (as an intrinsic factor) and can further vary according to extrinsic factors such as temperature, humidity, shading or weather conditions (Fritz *et al.*, 2000; Fritz *et al.*, 2003).

In this chapter an analysis of several important life history characteristics of the specialised gall fly *Urophora cardui* is presented. Factors investigated in the study population were body size, longevity, emergence rate and the temperature-dependence of larval development. These life history traits and their interrelationships form the background of the complex foraging behaviour of *U. cardui* females. Additionally they have an impact on foraging itself, since they are linked to larval performance.

2.1.2 Biology of the study system

The morphology of a plant gall induced by an insect is genetically determined by both, the plant and the insect (Béguinot, 2002; Weis *et al.*, 1988 and references within). Both participants of the study system used in this thesis are presented here in detail, first the host plant *Cirsium arvense*, then the gallfly *Urophora cardui*.

2.1.2.1 *Cirsium arvense*

Cirsium arvense L. (Scop.)(Asteraceae) (Fig. 2.1, Fig. 2.2) is a perennial plant (see Donald, 1994) characterised not only by high seed production, but also by extensive production of rhizomes (Peschken *et al.*, 1982). New adventitious shoots are formed on special root buds; thus a single plant is able to colonise a large area in a relatively short period of time (Donald, 1994; Peschken *et al.*, 1982). Shoots emerging in spring tend to elongate and form the well-known ramets, which may grow up to more than one meter height (Donald, 1994). The autumn shoots in contrast form rosettes, which can survive winter frosts. *C. arvense* is dioecious (see Donald, 1994). It is adapted to a broad range of soils: *C. arvense* can be found along small rivers as well as in dry fields with nutrient-poor conditions (Ang *et al.*, 1995; Donald, 1994; Edwards *et al.*, 2000). Especially under drought conditions *C. arvense* has a competitive advantage over annual plants, since it is able to extract water from greater depths in the soil (Donald, 1994). The root system is able to remain dormant for several years if the habitat is disturbed (Peschken *et al.*, 1982). The plant is very common in Europe as an ubiquitous weed and it has been introduced accidentally to Canada and the northern USA, where it causes heavy crop losses (Peschken & Harris, 1975; Peschken *et al.*, 1982).

Fig. 2.1: Young *C. arvense* seedlings in the green house.

Fig. 2.2: Several *C. arvense* ramets in a dense stand together with different grass species; one prominent *C. arvense* ramet is distinguishable at the front of the picture by its flower buds.

2.1.2.2 *Urophora cardui*

The Tephritidae are one of the larger dipteran families with approximately 4200 species in 500 taxa (Headrick & Goeden, 1998). *U. cardui* (L.)(Fig. 2.3) and the closely related East Asian *U. misakiana* are the only *Urophora* species, which form stem galls (Fig. 2.4). *U. cardui* occurs in the Palaearctic from Finland in the north (Jansson, 1992) to Greece in the south (Zwölfer, 1988) and from France in the west to near the Crimea (Zwölfer, 1982; Peschken & Harris, 1975) and Siberia in the east (Frenzel *et al.*, 2000; Korneyev pers. comm. to H. Zwölfer). However, its distribution is rather patchy (Seitz & Komma, 1984). At the margins of its distribution *U. cardui* switches to other host plant species (Frenzel *et al.*, 2000). In the eastern Mediterranean it attacks *Cirsium creticum* (H. Zwölfer pers. comm.) and in Siberia *Cirsium setosum* (Frenzel *et al.*, 2000). The adults hatch from galls induced in the previous year in early summer, at the end of May to early June (Lalonde & Shorthouse, 1985; Fig. 2.6). The male is about 5.5mm and the female about 6.5mm long (Peschken & Harris, 1975). Males and females can be distinguished by the visible oviscapt of the females (Fig. 2.3). Both sexes have characteristically banded wings, four dark broad bands are shaped in the form of a “W” (Headrick & Goeden, 1999). These play a significant role in mating displays (Peschken & Harris, 1975; Zwölfer, 1974b; chapter 3). The head of the flies is yellow, except for the dorsal side, which is black (Peschken & Harris, 1975).

Males of *U. cardui* establish territories on fully-grown thistles (Zwölfer & Schlumprecht, 1993). The reproductive and fighting success of the males appears to be correlated with the residence time of the males on the host plant (Babendreier & Hoffmeister, 1995), thus stressing the importance of the territory. The females visit several plants in their life and deposit eggs in the axillary buds of those thistles (Lalonde & Shorthouse, 1985; Fig. 2.6). When arriving on a new plant they first deposit eggs, up to over twenty in one bud (Freese & Zwölfer, 1996, own observations), before mating with the resident male (Fig. 2.6). Thus the thistle has not only a place for oviposition, but also the place for the adults to mate (Zwölfer, 1974a). It is known that the females of related species of the taxa *Rhagoletis* and *Dacus* require repeated mating in order to maintain a high level of fecundity (Fletcher, 1989) and it has been assumed that this holds true for all fruit fly species (Fletcher, 1989). Although the females occur often in high density plant stands, they search for a very long time (Freese & Zwölfer, 1996) before accepting a plant. Even on an acceptable plant they often need up to three hours until they decide on one particular axillary bud or move on to another plant in the patch (own observations, see chapter 3). The broad variability in *U. cardui's* clutch sizes and numbers of cells per gall can be explained by differences in the quality of the oviposition sites and providing an explanation for female host plant discrimination (Freese & Zwölfer, 1996).

Fig. 2.3: Two females of *U. cardui* and one male (left) on their host plant, the individuals have been marked to enable observer discrimination (see part 2.2).

Fig. 2.4: Three-week old growing gall of *U. cardui* on a side shoot of *C. arvense.*

The main predators of the adult flies are spiders, particularly two *Theridion* species that live on the plants and need only a few seconds to localise and catch a fly landing on the plant (Rauh, 1994).

The larvae are attacked by several parasitoids, particularly two *Eurytoma* species (Hymenoptera: Eurytomidae): *E. serratulae,* which is an internal parasite and *E. robusta*, which is an external parasite (Schlumprecht, 1989; Zwölfer, 1979). These can cause larval mortality rates up to 100% per gall (Schlumprecht, 1990; Zwölfer, 1979; Zwölfer & Arnold-Rinehart, 1993). Two other less common ecto-parasitoids in the region around Bayreuth are *Torymus chloromerus* (Hymenoptera: Torymidae) and *Pteromalus elevatus* (Hymenoptera: Pteromalidae) (Redfern, 1983).

Unlike all other species of the genus *Urophora*, *U. cardui* and its sister taxon *U. stylata* do not have three free-living larval stages (Headrick & Goeden, 1998). In other *Urophora* species the first larval stage (L1) leaves the egg and starts gall induction, whereas in *U. cardui* and *U. stylata* the first larval stage remains in the egg (Lalonde & Shorthouse, 1984; Lalonde & Shorthouse, 1985; Shorthouse, 1986). The eggs of *U. cardui* hatch 7-10 days after oviposition (Jansson, 1992). The gall is usually many-chambered (Fig. 2.5), each chamber containing a single larva (Lalonde & Shorthouse, 1984; Lalonde & Shorthouse, 1985; Fig. 2.5). Between one and more than twenty larval chambers have been found in one gall (Freese, 1997 own observations). The number of chambers and thus larvae per gall does have an influence on larval survival (Freese & Zwölfer, 1996) but not on the longevity of the adults (Peschken & Harris, 1975). One of the main larval mortality factors, apart from parasitoids, is egg mortality (Freese, 1997).

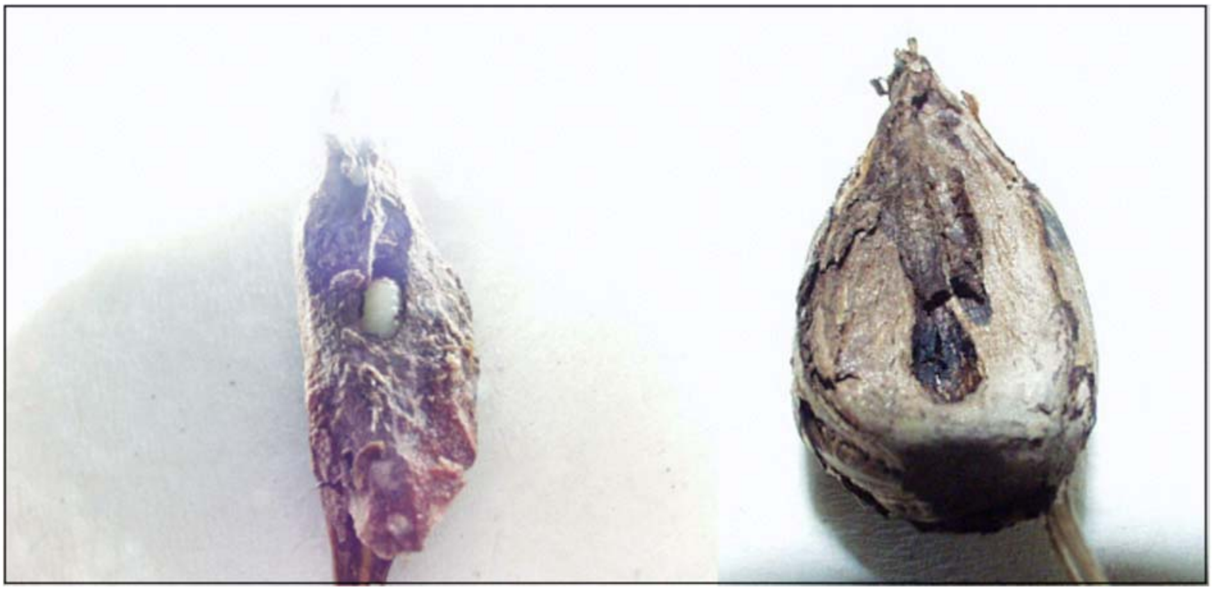

Fig. 2.5: Left: longitudinal section of a gall of *U. cardui* containing one barrel shaped larva in its chamber, right: longitudinal section of a gall of *U. cardui* with two larval chambers.

The second instar larvae that leave the egg do not feed very much, but initiate gall formation (Headrick & Goeden, 1998; Lalonde & Shorthouse, 1985; Fig. 2.6) using substances, mostly cytokinins, from their salivary-glands (Leitch, 1994; Sakuth, 1996). After 12-20 days, the development of a gall becomes visible. The first patches of primary nutritive cells appear during the growth phase, but these are seldom consumed (Lalonde & Shorthouse, 1985). The third instar larvae start to feed on the ample secondary nutrient cells of the gall tissue (Lalonde & Shorthouse, 1982; Lalonde & Shorthouse, 1985; Shorthouse, 1986). Gall growth takes place for about 30 days, and is terminated by the formation of woody and hard tissue (Lalonde & Shorthouse, 1984). This last growth phase, also named the maturation phase, is characterised by lignification of thick layers of the larval chambers and the gall parenchyma (Lalonde & Shorthouse, 1982; Lalonde & Shorthouse, 1984; Fig. 2.6). Towards the end of larval development the entrances of the corresponding feeding tunnels are filled with callous plant tissue (Redfern, 1983). *U. cardui* hibernate as third instar larvae, and pupate in March and April (Lalonde & Shorthouse, 1982; Redfern, 1983). Due to the increase in humidity in spring the callous tissue at the entrance of the larval feeding tunnels is partly decomposed and thus air diffuses into the gall (Lalonde & Shorthouse, 1982). This process induces the emergence of the adults in May/June (Redfern, 1983; Fig. 2.6).

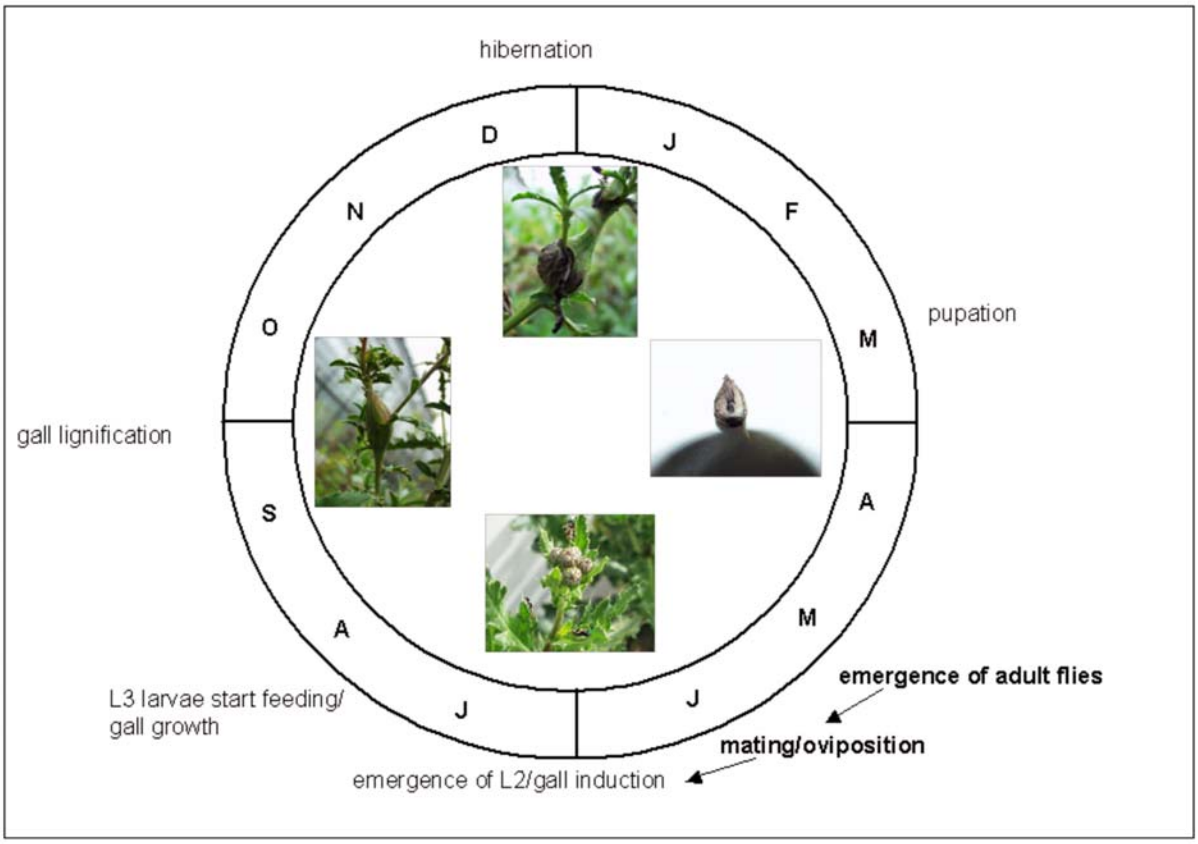

Fig. 2.6: Schematic drawing of the life cycle of *U. cardui*. In the centre four important life cycle stages are shown. The circle symbolises the year with the letters indicating the twelve months. The different life cycle steps are arranged around the circle. Bold: important steps for the present investigation.

In a variety of herbivorous species, the larvae are able to change the nutrient composition of the tissue (Hartley, 1998; Hartley & Lawton, 1992). Larvae of galling-species induce a strong sink for carbohydrates and nitrogenous compounds in the plant (Bagatto & Shorthouse, 1994; Larson & Whitham, 1997), this is true for *U. cardui* also (Gange & Nice, 1997). Due to the high complexity of the gall, the larvae are able to recruit nutrients from the whole plant (Shorthouse, 1986). Nevertheless *U. cardui* exerts only limited influence on the plant's fecundity (Lalonde & Shorthouse, 1985), as common with most other gall forming dipteran species (Brewer *et al.*, 1987 and references within). By attracting and consuming nutrients, galls redirect plant resource allocation from shoot growth and presumably also rhizome formation (Gange & Nice, 1997). However, due to alleviation of sink limitation the formation and growth of galls may result in increased rates of photosynthesis (Fay *et al.*, 1996).

In *U. cardui* water shortage leads to a high proportion of larval death (Rotherey, 1986; Zwölfer, 1994), which demonstrates a high requirement for water and soil-born nutrients (Gange & Nice, 1997). *U. cardui* evolved on thistles in riverine forests and banks in shaded places (Zwölfer, 1978; Zwölfer, 1982; Zwölfer, 1988; Zwölfer, 1990; Zwölfer, 1999). This habitat preference is due to several factors. *C. arvense* can persist as shoot system for several years, if the conditions are unfavourable, while *U. cardui* needs oviposition places every summer. Additionally the stomata on the gall surface cannot be closed (Lalonde & Shorthouse, 1984). Thus in open, sunny localities, such as thistles growing along agricultural field edges, there is an increased risk that the galls dry out, causing developmental failure, unless there is sufficient shading from trees (Zwölfer, 1994). Long-lived populations of *U. cardui* exist mainly in relatively moist sites, such as riverine forests, partly flooded sandpits or similar habitats with a high water supply (Zwölfer, 1988; Fig. 2.7, Fig. 2.8). Around Bayreuth *U. cardui* occurs in nearly all stands of *C. arvense*, which have a good water supply and/or shading from trees like at wood and forest margins. *U. cardui* usually exists in quite small subpopulations, the mortality inside such a community can reach 100% (H. Zwölfer pers. comm.) One of the reasons for high extinction rates is the ectoparasitoid *E. robusta* (Eber & Brandl, 1994). One could describe the population structure of *U. cardui* as metapopulational: a large number of small subpopulations, which go extinct and are recolonised readily, the occurrence of *U. cardui* in one host plant stand is coupled with high extinction and re-immigration rates.

Fig. 2.7: One habitat of *U. cardui* in a sandpit with several temporary ponds. This location is near Bayreuth, neighbouring the village of Großweiglareuth.

Fig. 2.8: A closer look at the above-mentioned habitat, showing one host plant stand.

It was attempted to use *U. cardui* as a biological agent against *C. arvense* in Canada (Lalonde & Shorthouse, 1984) as its high specialization might make it suitable for biological pest control (Zwölfer & Harris, 1971). *U. cardui* was successfully introduced to Canada (Peschken & Derby, 1997), but in contrast to the success of other species in biocontrol of weeds, these species can be found on 80-100% of the plants in one habitat, *U. cardui* occupies only 1% of the host stands (Zwölfer, 1994). From this time on, interest in this species increased and several studies concerning its behaviour, life history, dispersal and habitat choice (Jansson, 1992; Schlumprecht, 1989; Schmidt, 1994; Schlumprecht, 1990 and references within, Zwölfer & Schlumprecht, 1993; Zwölfer, 1982) as well as gall morphology (Lalonde & Shorthouse, 1982; Lalonde & Shorthouse, 1985; Redfern, 1983; Shorthouse, 1986) were initiated to clarify the reasons for the low rate of host plant usage.

2.2 Material and Methods

2.2.1 Origin of plants and flies, general rearing

Thistles and rhizomes were collected in early spring, when the first leaves of the plants were visible. Different habitats were used for plant collection: a wet meadow, a large border of a crop field and the bank of a brook. Several preliminary experiments showed that the females did not discriminate between plants of different origin, thus the results of plants from different origins were pooled in all experiments.

Thistle growing and synchronization with emergence of the flies was performed with a similar breeding strategy as used by Sakuth (1996).

Plants were potted in soil (5 parts neutral humic soil, 2 parts pumice, 2 parts lava, 1 part loess, 2 parts peat and 2500g Osmokote^{TM} (a long-time fertilizer)/1m^3 soil)) and kept in the open to provide semi-natural conditions. In autumn and winter plants were grown in the greenhouse at (L:D 15 (23°C):9 (18°C)). The light conditions were achieved by an array of mercury lamps (Osram Power Star HQ I–TS 250 WINAL with a minimal light intensity of 400µEm^{-2}s^{-1}). The plants were watered twice a day to avoid drought stress, which would have affected female preference and larval performance. The thistles were repotted to larger pots after approximately two weeks growing and again two to four weeks later. The final pot size was three or five litres, according to plant size. Each plant was numbered, the height measured, and axillary buds and flower buds were counted before usage. The axillary buds were counted from the oldest leaves up to the top of the plant and numbered in the same direction. For multi-stemmed plants only the height of the largest shoot was recorded, but total shoot number was noted and all buds were counted as well. No thistle was used twice.

The *U. cardui* flies originated from several small populations within approximately twenty kilometres around Bayreuth, Germany. Some of the flies were part of the newly established laboratory population, although this experimental population could not be maintained without new flies from the field. The galls were collected in late summer and autumn and stored in the refrigerator until initiation of eclosure.

The flies emerged from these galls in Petri-dishes (ø 14 cm) and were then marked individually with a two colour-code (Hobbyline, Acryl - Colours in light green (10), blue (24), red (15), yellow (03) and orange (04)) on the wings. No interference of the marking with the behaviour was observed in preliminary comparisons. The same colour codes were used again, if new individuals emerged from galls after the original possessor had died. In total 294 males and 271 females were used.

Between the experiments the flies were held in plastic cages (volume: 6l, Fauna box by Savic, Belgium) lined with regularly moistened cellulose tissue (Fig. 2.9). They were fed *ad libitum* with 50% honey in water solution or sucrose-solution 67.5% (w/v) (Freese & Zwölfer, 1996; McCaffrey *et al.*, 1994). Water was also provided *ad libitum*. A maximum of 15 flies was kept per box. Two of the boxes were for single sexes, containing only males or females, to have a stock of virgin, naïve individuals. In most cases prior to experiments the flies were not confronted with thistles, in order to enhance individual motivation.

Fig. 2.9: One of the plastic cages for flies containing a vial with water.

2.2.2 Investigation of life history parameters

Some general data for each emerging fly were noted. They were weighed (Sartorius balance, Sartorius Germany, accuracy: 0.1mg) immediately after eclosure and immediately after death, if possible. Of a randomly chosen sample of males and females the head capsule width was measured with a calibrated ocular micrometer with a binocular microscope (Wild M38, Heerbrugg, Switzerland) at 16-fold magnification. Longevity of males and females was analysed in relation to body size and sex.

Additionally the emergence rate in relation to the collection-date and the duration of gall storage in the refrigerator were analysed. The data presented here were obtained from 102 galls, which were part of my established experimental population. Data from galls collected in nature were not analysed in relation to storage in the refrigerator, since the potential presence of parasitoids had a substantial impact on the emergence rate, which might have hidden influences of gall-storage duration on the emergence rate.

Galls were either stored in the refrigerator or immediately put into Petri-dishes for adult emergence according to procedure in individual experiments. The minimum time period for galls in the refrigerator was one week and the longest was 28 weeks. According to duration of storage in the refrigerator, galls were separated in five groups (Table 2.1).

Table 2.1: Classification of *U. cardui* galls with respect to the duration of storage in the refrigerator.

Class	Weeks of gall storage	Number of galls per group
0	0 weeks	17
1	1-4 weeks	22
2	5-8 weeks	11
3	9-16 weeks	13
4	> 16 weeks	36

2.2.3 Statistics

All raw data were organized in an Access-database (Access 2000, Microsoft corporation). Preliminary table sorting and analyses were carried out in Access and in Excel (Excel 2000, Microsoft corporation); statistical analysis was performed in SPSS 10.0 (SPSS Inc.). Graphs were also executed in SPSS. Data sets, which were not normally distributed were either transformed or an appropriate non-parametric test was used. Before employment of a t-test a Levene-test of homogeneity of variances was performed.

2.3 Results

2.3.1 Weight at eclosure

The 271 females had an average emergence weight of 10.67mg (±2.79; Fig. 2.10), while the 294 males weighed on average 9.9mg (±2.75; Fig. 2.11). Thus the females of the experimental *U. cardui* population were significantly heavier than males at eclosure (t-test: T=-3.397, df=563, n=565, p=0.001; Levene test: F=1.103, p=0.294).

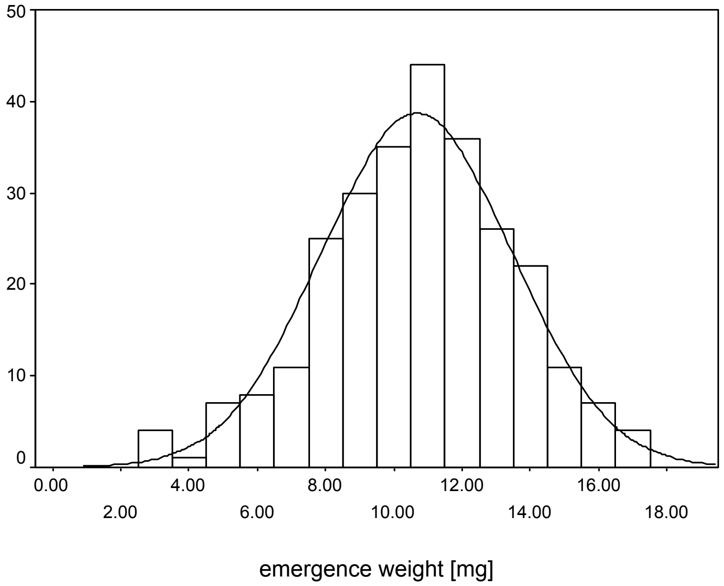

Fig. 2.10: Histogram showing the frequencies of the weight at eclosure of the females [mg]. Overlaid curve=normal distribution.

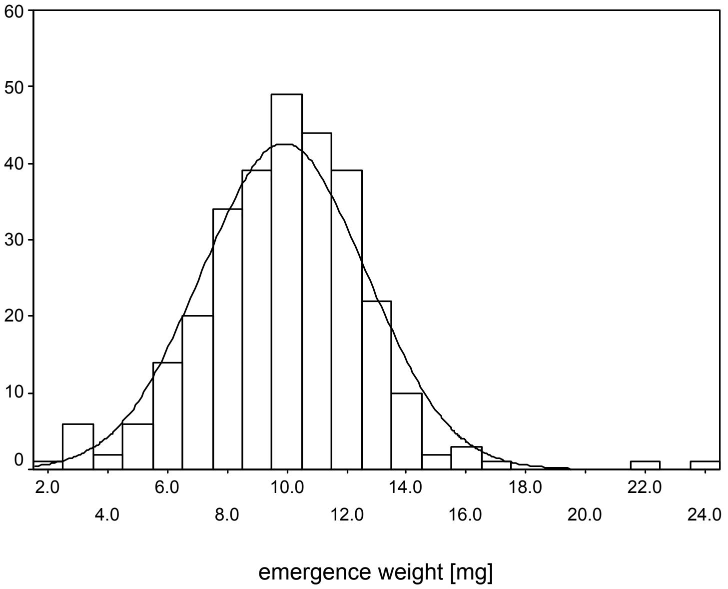

Fig. 2.11: Histogram showing the frequencies of the weight at eclosure of the males [mg]. Overlaid curve=normal distribution.

2.3.2 Weight at the time of death

The weight at death (henceforth referred to as death weight) of 144 females was estimated. It was measured directly after the death of the individual and is thus the fresh weight. On average it was 7.68mg (±3.01; Fig. 2.12). The mean death weight of 169 males was 6.84mg (±2.84; Fig. 2.13). As at eclosure, at the time of death the females of *U. cardui* were heavier than the males, although the difference was smaller (t-test: T=-2.534, df=311, n=313, p=0.012; Levene test: F=1.09, p=0.297).

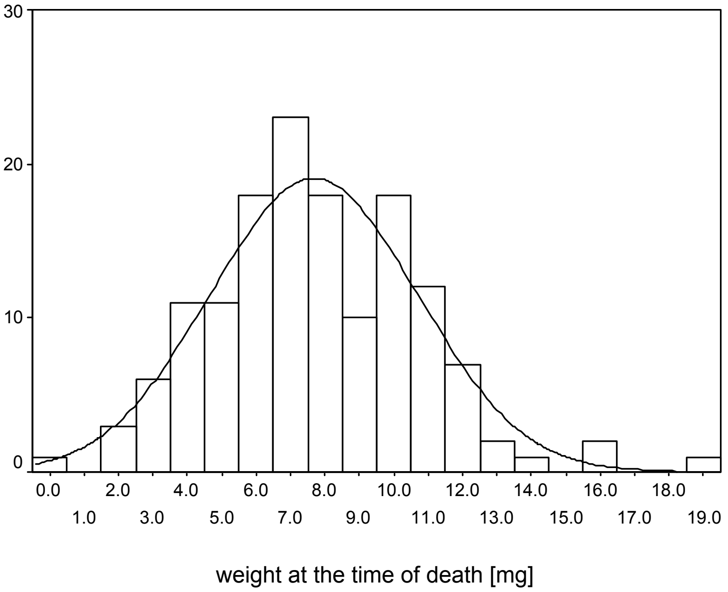

Fig. 2.12: Histogram showing the frequencies of the weight [mg] at death of 144 females. Overlaid curve=normal distribution.

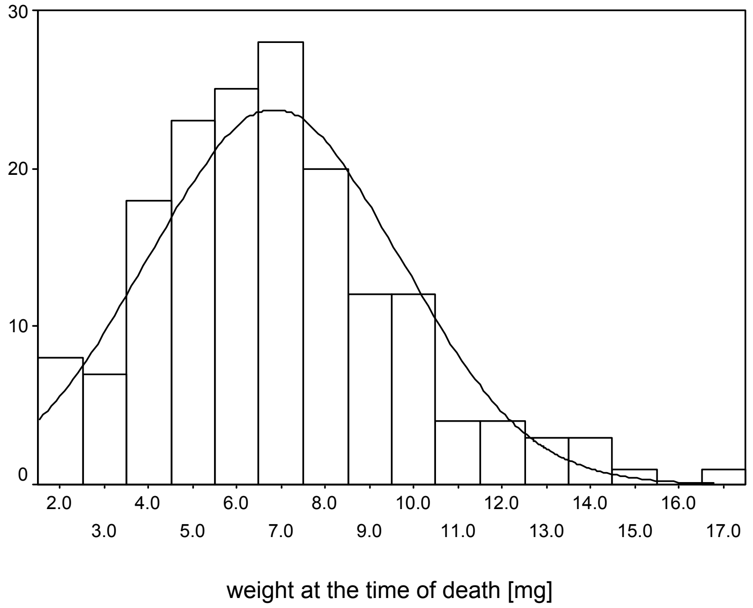

Fig. 2.13: Histogram showing the frequencies of the weight [mg] at death of 169 males. Overlaid curve=normal distribution.

2.3.3 Head capsule width

The head capsule width of 74 females and 63 males was measured, in order to test if it may also be used as an indicator of individual size, as body weight generally is. On average the width of female capsules was 1.65mm (± 0.3mm; Fig. 2.14) and that of the males 1.67mm (±0.15mm; Fig. 2.15). There is no significant difference between the head capsule width of males and females (Mann-Whitney U-test: Z=-0.359, df=1; n=137, p=0.72).

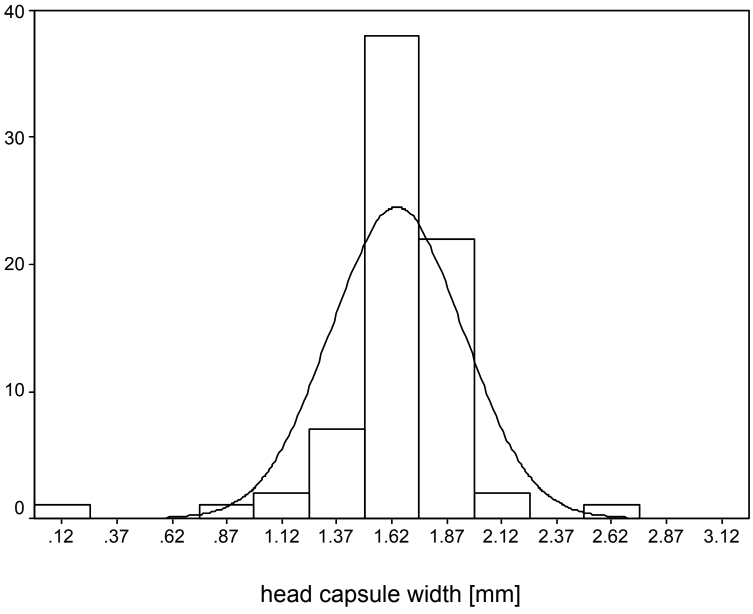

Fig. 2.14: Frequencies of the head capsule width [mm] of 74 females. Overlaid curve=normal distribution.

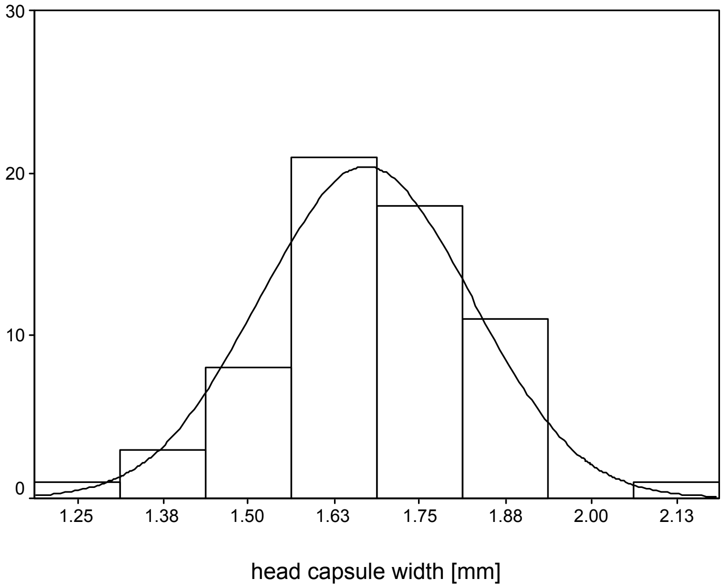

Fig. 2.15: Frequencies of the head capsule width [mm] of 63 males. Overlaid curve=normal distribution.

2.3.4 Relationships between these measures of individual size

Weight at emergence and at death are significantly correlated, both in females (R_S=0.524, df=1, n=144, p<0.001) and males (R_S=0.392, df=1, n=169, p<0.001). Emergence weight and head capsule width are also significantly correlated in both sexes ($R_{S\ females}$=0.598, df=1, n=73, p<0.001; $R_{S\ males}$=0.537, df=1, n=63, p<0.001). These results are summarized in Table 2.2.

Table 2.2: Summary of the correlations between the different scales of individual size: emergence weight, death weight and the head capsule width of males and females of *U. cardui.*

		females			males		
		emergence weight	death weight	head capsule width	emergence weight	death weight	head capsule width
emergence weight	n		144	73		169	63
	R_S		0.524	0.598		0.392	0.537
	p		< 0.001	< 0.001		< 0.001	< 0.001
death weight	n			44			41
	R_S			0.375			0.418
	p			0.012			0.007

It is remarkable that the correlations between the different scales of body size are not correlated with each other to a higher degree. There is a high variability, as shown for example for the correlation between emergence weight and head capsule width of the males (Fig. 2.16). Regarding emergence and death weight they are even significantly different within both sexes (Wilcoxon test $_{\text{females}}$: Z=-9.571, df=1, n=144, p<0.001, Wilcoxon test $_{\text{males}}$: Z=-9.904, df=1, n=169, p<0.001). At death nearly each individual is lighter than at eclosure. This was expected, in the females weight loss may mainly be due to egg loss, in both sexes degeneration of tissues leads also to weight loss.

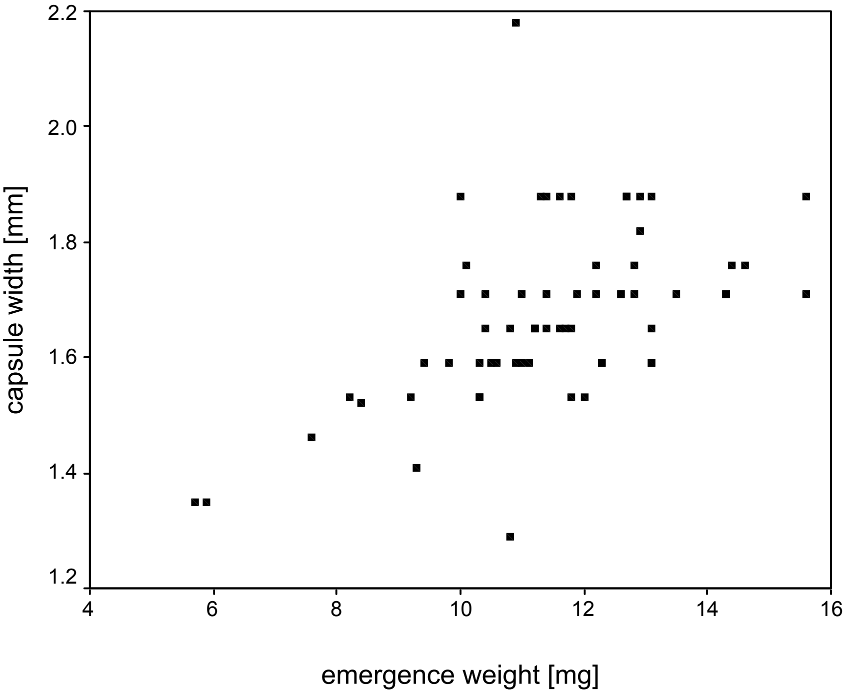

Fig. 2.16: Scatter plot of the correlation between male emergence weight [mg] and their head capsule width [mm] (R_S=0.537).

2.3.5 Longevity of males and females

Of 235 females kept under the experimental conditions the longevity could be determined. They lived for a median of 7 days (quartiles: 3-11; Fig. 2.17). In contrast males lived in the median for 5 days (quartiles: 3-9.5, n=265). A Mann-Whitney U-test revealed, that females lived significantly longer than males (Mann-Whitney U-test: Z=-2.222, df=1, n=500, p=0.026). But as Fig. 2.18 shows the difference is small, since there are many extreme values. There was no correlation between longevity of females and their weight at eclosure as a scale of body size (R_S=-0.038, df=231, n=232, p=0.561). The same applies for the males (R_S=-0.054, df=263, n=264, p=0.384).

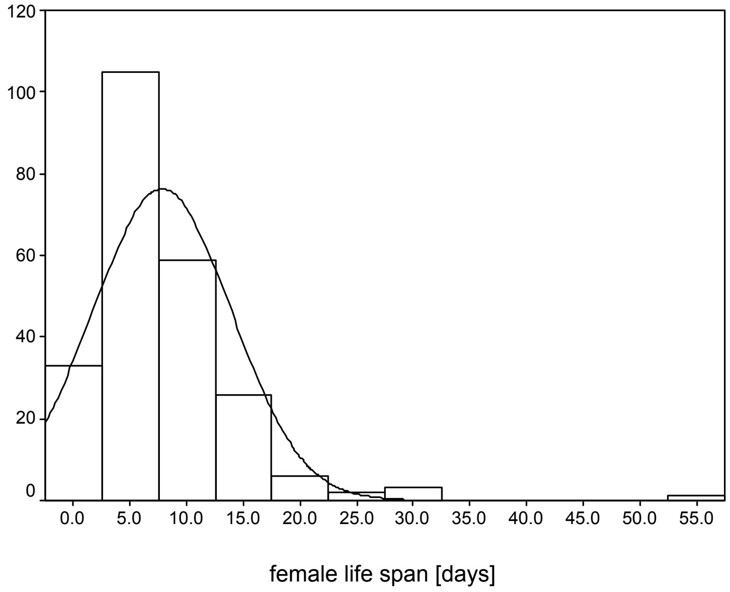

Fig. 2.17: Histogram of female longevity. Overlaid curve=normal distribution.

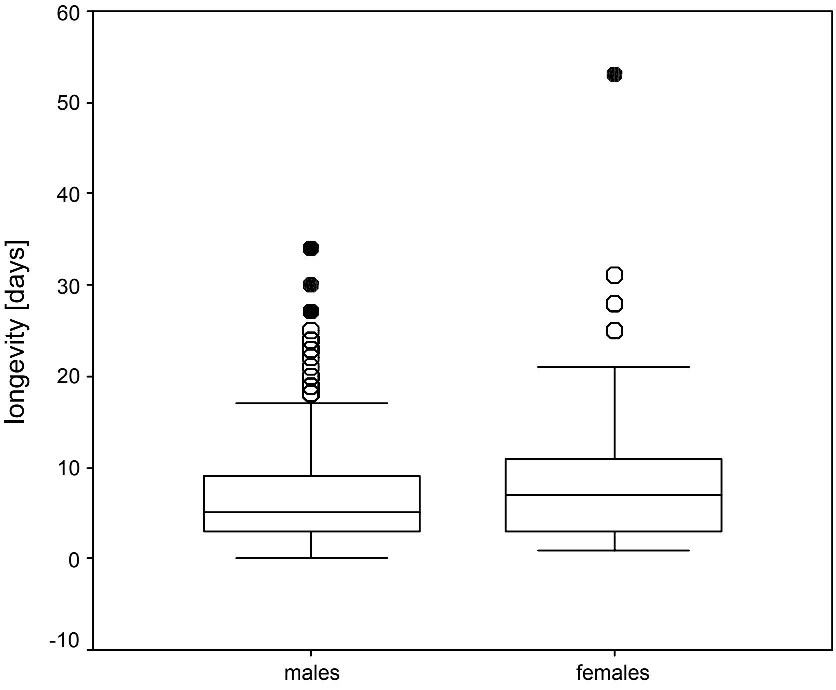

Fig. 2.18: Longevity of male and female *U. cardui.* Lines in the boxes=median; boxes=25-75%; whiskers=minimum and maximum; open circles=outliers (1.5-3 times inner-quartile distance); circles=extreme values (>3 times inner-quartile distance).

2.3.6 Emergence rate

101 adults emerged from 71 of the 102 galls, which developed on the thistles of the laboratory population. The median number of larvae per gall was 3 (quartiles: 1-5; Fig. 2.19). One adult (median) emerged out of each gall (quartiles: 0-2; Fig. 2.20). The number of adults divided by the number of larvae gave the average rate of survival during the larval state (33.33 % of the larvae (median) in a gall emerged (quartiles: 0-100%)).

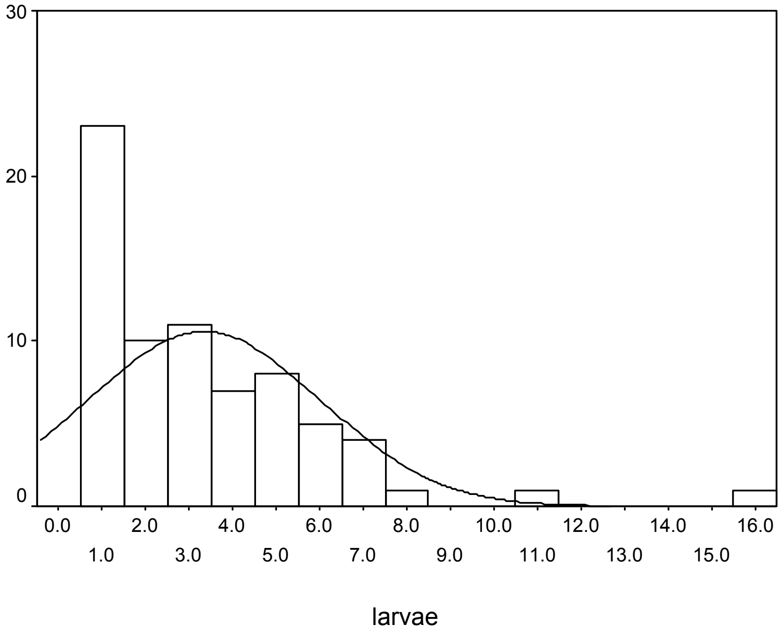

Fig. 2.19: Frequencies of the number of *U. cardui* larvae per gall. n=102 galls; overlaid curve=normal distribution.

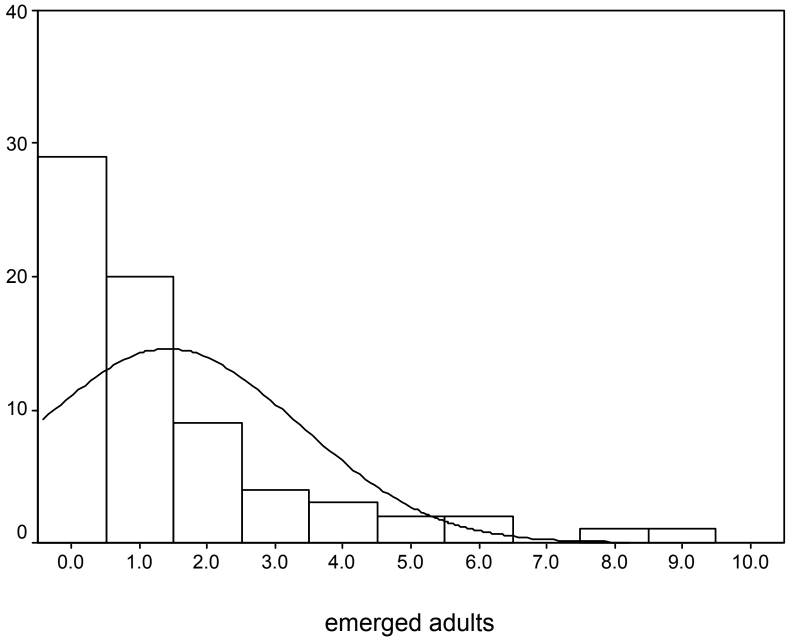

Fig. 2.20: Frequencies of the number of adults of *U. cardui* that emerged from one gall. n=102 galls; overlaid curve=normal distribution.

If the number of emerged adults per gall is compared to the number of the larvae, which had been in that specific gall, the distribution is bimodal (Fig. 2.21). A high larval mortality was evident. Additionally the bimodal distribution hints that the emergence rate might be higher when there were more larvae in a gall.

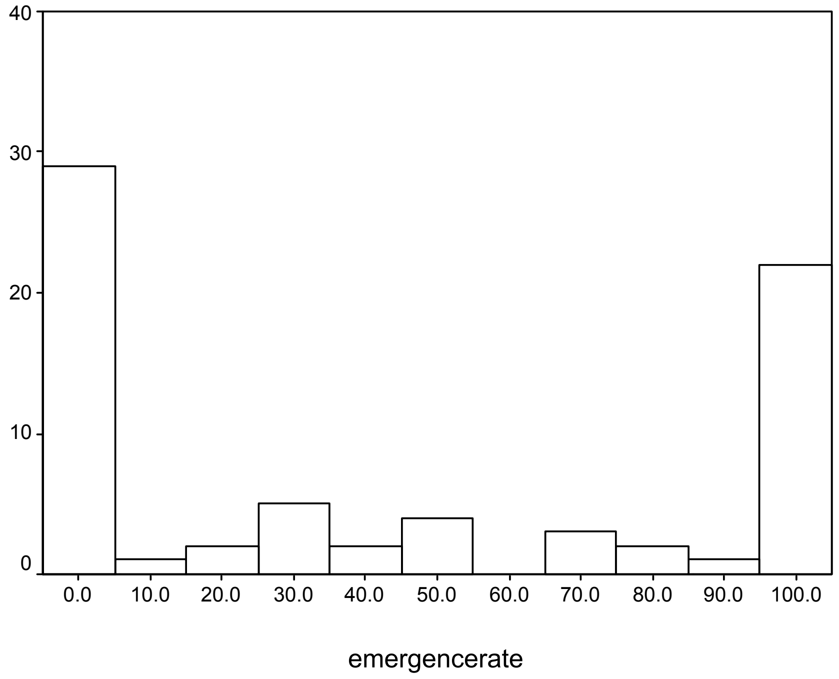

Fig. 2.21: Frequencies of the percentage of the emergence (number of emerged adults/number of larvae *100).

Duration of cold storage prior to emergence was not correlated to the proportion of larvae, which were able to emerge successfully (R_S=0.024, df=4, n=71, p=0.841; Fig. 2.22). The same applies if the number of weeks of cold storage is directly correlated to the emergence rate (R_S=0.061, df=69, n=71, p=0.614). Accordingly there is no general difference in emergence rate between galls stored in the refrigerator or galls, which were opened immediately without a cold storage (Mann Whitney U-test: Z=-0.226, df=1, n=71, p=0.821).

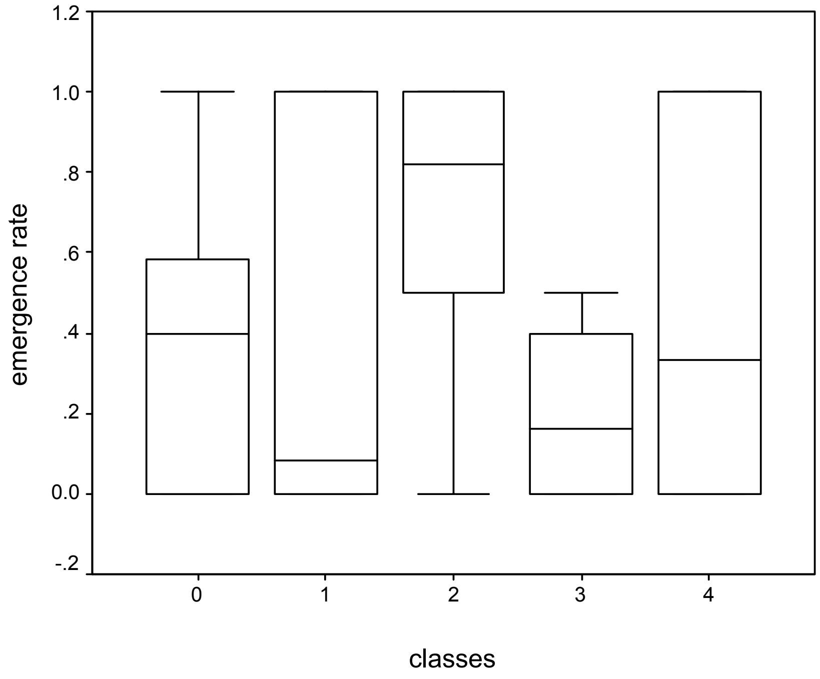

Fig. 2.22: Emergence rate related to the number of weeks for which the galls had been stored in the refrigerator. The definition of the classes is given in Table 2.1. Lines in the boxes=median; boxes=25-75%; whiskers=minimum and maximum emergence rate.

2.4 Discussion

Females of *U. cardui* were heavier than the males in the experimental population. In contrast the head capsule width of both sexes was comparable. It is remarkable that the different representative parameters to describe body size used here, were only slightly correlated with each other. Death weight was significantly lower than the weight of the animals at eclosure. This might indicate the usage of a high amount of energy, which seems not to be replaced during their lifetime as adults (Strohm & Daniels, 2003; Wilps & Collatz, 1986). The weak correlation between emergence and death weight may also be due to a variation in the life-span of the adults, which would produce a higher discrepancy between the two measurements if the weight loss is constant over the life-span (Wilps & Collatz, 1986). However, the difference between the weight at eclosure and the death weight does not explain why the correlation between weight and head capsule width was only low (Klingenberg & Spence, 1997). The weight at eclosure was treated as a better representation of the nutrients the adults possess at eclosure, as it is not influenced by later living conditions. Thus death weight was not used further as an indicator of body size. If necessary, weight at eclosure and head capsule width were both treated as scales of body size in further experiments.

As one factor of individual fitness the longevity of an individual should be correlated with the body size (Klingenberg & Spence, 1997; Larsson & Tengö, 1989; Strohm, 2000; Tepedino & Torchio, 1982). One common scale of body size is the weight at eclosure, since it indicates the amount of resources available for foraging, mating, oviposition, longevity and other traits of adult life (Larsson & Tengö, 1989; Strohm & Daniels, 2003). In *U. cardui*, male and female longevity was not correlated with body size. This is an important and interesting result for a variety of reasons, first body size is considered to be the life history character with the highest impact on dispersal and also on mating strategy (Krainacker *et al.*, 1989). Second, it is a topic, which remains controversial in the literature (Alcock, 1979; Gaillard *et al.*, 2000; Honêk, 1993; Kim, 1997; Klingenberg & Spence, 1997; Smith & Fretwell, 1974; Strohm & Daniels, 2003). Nevertheless for several other fruit fly species such as *Ceratitis capitata* and *Dacus dorsalis* no relationship between the size class of the larvae and their longevity as adults has been found (Krainacker *et al.*, 1989). It can be assumed, that larval and adult size is correlated. As far as I know there are no further studies on other fruit fly species available, but there exist studies concerning longevity in relation to diet for example in other *Urophora* species (McCaffrey *et al.*, 1994). If *U. affinis* and *U. quadrifasciata* are supplied with honey and sugar water the adults reach a mean life span of 30 days. Nourishment with yeast hydrolysate resulted in a shorter life span (12-14 days), while individuals provided only with pure water lived for 8-10 days (McCaffrey *et al.*, 1994). Compared to the effect of nourishment with yeast hydrolysate this is surprisingly long. It is thus important to mention, that McCaffrey *et al.* (1994) held the flies captive in a quiescent state, while the flies of the present study were engaged in several activities, including flying, copulation and egg-laying. The flies in the present study thus needed more energy and had a shorter life-span compared to those of McCaffrey *et al.* (1994). In their study both *U. affinis* and *U. quadrifasciata* males lived longer than the females, which is in contrast to this study of *U. cardui*, where the males died earlier than the females. As in *U. cardui,* females of *U. jaceana* and *U. quadrifasciata* were heavier than the males (Burkhardt, 1999). Leather (1990) found no relation between weight and longevity but a significant relationship between longevity and the availability of an adult food source in *Pieris flammea* (Lepidoptera: Pieridae). As observed in the rearing containers, *U. cardui* adults do not feed very much; therefore I assume that the larval food source may also be of a high importance for determination of the fecundity and longevity of the adult flies.

Emergence rate was not influenced by application of cold periods of different length. Duration of development was independent of presence or the duration of an interruption in form of a cold period. Flies were always predictable in date of emergence. Thus the period of cold temperature only interrupts development but has no further influence on emergence and the speed of development after the cold period. This is in contrast to the influence of a cold period on the larvae of *Eurosta solidaginis* (Diptera: Tephritidae). Mild winters reduced larval survival and the fecundity of emerging adults (Irwin & Lee, 2000). In the following chapters data from flies with different periods of cold storage were pooled.

The life history characters laid out here have an impact on the complex foraging strategy of the specialised gall fly *U. cardui*. Females should select oviposition sites, which guarantee a high larval performance, an adaptive syndrome (Price, 1994) that represents one of the main selective powers on the evolution of the emergent properties of physiology (Davis, 1984), morphology (Scudder, 1961; Vilhelmsen *et al.*, 2001) and behaviour (Bush, 1969; Craig *et al.*, 1989; Fletcher & Prokopy, 1991; Fritz *et al.*, 2000).

2.5 Acknowledgements

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3 Behavioural aspects of *Urophora cardui* (Diptera: Tephritidae) in relation to the host plant *Cirsium arvense* (Asteraceae)

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The analysis of behaviour of a specialised herbivorous insect on its host plant hints to cues of the host plant important in selection for oviposition sites. Experiments using artificial or modified plants show the rigidity of the host plant template employed in selection. The behaviour of males and females of the specialised gall fly *Urophora cardui* were recorded on its host plant *Cirsium arvense* as well as on modified plants. Different behaviours were described and defined.

While females concentrated their movement towards the middle and the top of the host plant, where the existence of axillary buds suitable for oviposition is most likely, males moved around the whole plant, although marking was often restricted to upper leaves and the stem. On natural host plants, both sexes showed a high individual variability with regard to the time spent on different behaviours. Females spent most of the time on running on the plant, running, resting and in copulation, while the time budget of the males was dominated by copulation.

In females there existed one behavioural loop on natural host plants between probing and oviposition. In *U. cardui* males close relations between threatening, fighting and running on the plant, and between marking, grooming and resting existed.

Only few animals remained long on modified host plants. If the host plant was modified with nail polish, male behaviour was affected more markedly than that of the females. Individuals of both sexes restricted their movement to smaller areas, but in the case of the males nearly all movement around the whole plant was suppressed. Most individuals of both sexes left modified host plants nearly immediately. This behaviour indicates the presence of a fairly rigid host plant template, which is used in host plant search, recognition and selection. On modified plants the time spent on individual behaviours was more uniform in both sexes.

3.1 Introduction

For a better understanding of Tephritid foraging strategies (including foraging for larval host plants and territories), it is crucial to observe their behaviour in relation to their natural host plants (Raghu *et al.*, 2002; Roitberg, 1990). Almost no data are available on the behaviour of fruit flies under natural conditions; in the field tracking adult flies is very difficult (Aluja & Prokopy, 1992; Burkhardt, 1999). In the laboratory they can be observed in a semi-natural environment, in which their behaviour is assumed to be similar to that under natural conditions (Aluja *et al.*, 1989; Fletcher & Prokopy, 1991; Prokopy & Roitberg, 1989; Roitberg, 1988). Nevertheless caution is required when extrapolating the results of laboratory studies to the natural situation (Roitberg, 1988).

The foraging behaviour of female fruit flies is expected to be optimal with respect to selection of suitable larval host plants (Craig *et al.*, 1989; Jaenike, 1978; Pyke, 1984). Females of galling insect species should be especially selective, since the larvae are not able to compensate for poor female choice, as they are not able to leave the plant organ selected for by their mothers (Ballabeni *et al.*, 2001; Craig *et al.*, 1989). The thistle stem gall fly *Urophora cardui* analysed here, is specialised on the creeping thistle *Cirsium arvense* (Zwölfer & Schlumprecht, 1993; Zwölfer, 1978; Zwölfer, 1982). The larval host plant is the centre of activity for the adult flies (Zwölfer, 1974a): the males use the plant as a territory for courtship and mating (Babendreier & Hoffmeister, 1995; Frenzel *et al.*, 1990) and the females of *U. cardui* visit the larval host plant in order to oviposit and to mate (Peschken & Harris, 1975; Zwölfer, 1978). Individuals of both sexes are assumed to possess a template (also defined as search image, (see Krebs & Davies, 1993; Tinbergen, 1934) of their host plant allowing them to recognise it among a multitude of alternative plants. As a first step in the analysis of the complex foraging strategy performed by males and females of *U. cardui*, detailed behavioural observations were conducted.

3.1.1 Definitions of the behavioural traits performed by *U. cardui*

In the following, short descriptions of the different behaviours performed by both sexes are presented and terms for these respective behaviours defined. These definitions of the different behaviours are used here and in all subsequent chapters. Adults of *U. cardui* were observed in semi-natural cages on larval host plants, *C. arvense* (chapter 3.2).

3.1.1.1 Behaviours performed by both sexes

Running:

Individuals of *U. cardui* walk on the ground, the walls or the top of the observation cage. Especially when approaching an object, the individual stops for a few seconds, erects its head and thorax and moves its antennae very rapidly in all directions. This seems to be a kind of orientation behaviour. In the observations this presumed orientation behaviour was not recorded separately, since it lasted a very short time, and seemed to be related to movement rather than to other decisions. It is described for related species in detail by Burkhardt (1999). Running arises in part as a consequence of confrontment in the observation cages. In nature it may correspond to movement on the ground, but its actual occurrence in nature with densely growing shrubs is unknown (chapter 2.1.2).

Flying:

Short distance movement of *U. cardui* males and females is often achieved by short flights. This can be movement to another plant, the walls or the top of the cage, or to another leaf or the stem of the same plant. This flight to other locations on the same host plant was of most frequent occurrence. Before take-off, most flies showed the aforementioned presumed orientation behaviour. It is not possible to give duration for most of the flights, since they lasted less than a second and were thus unmeasurable. Therefore flight is recorded in the ethograms, but not in any of the time–comparisons.

Running on the plant:

The fly moves around the stem and/or the leaves of the thistle. It only stops for maximally five seconds. With this behaviour the aforementioned presumed orientation behaviour also occurs, especially before short flights or moving to another leaf or the stem.

Resting:

The individual stays at one fixed position for at least five seconds, without grooming, running on the plant or other activities. Occasionally it turns round very slowly. This always occurred without a change of resting place, therefore this behaviour was not recorded separately. It seems to be related to resting, but the function of it remains unexplained. It may also be a kind of orientation behaviour.

Grooming:

The male or female grooms itself for at least five seconds, rubbing its antennae, its head, the abdomen or the thorax with its legs. The legs are rubbed against each other as well. This behavioural sequence resembles the grooming behaviour of the housefly *Musca domestica* (Diptera: Muscidae), which is readily observed during summertime.

Copulation attempts/Copulation trial:

Copulation is often preceded by a short courtship period, in which the male slowly approaches the female (see chapter 3.1.1.3). In approximately half of all copulation attempts and copulations, which were observed from the start, the male approached the female from behind and tried to jump onto the female, often without success. Most males tried to jump on the same female several times. If, after several attempts of the male, the female remained unreceptive, it tried to avoid the male’s hind legs trapping her ovipositor. It would also try to shake off the male or to fly away immediately. In some cases the male was nonetheless successful, and copulation took place. Copulation attempts can be very short, about half a minute, or last up to ten minutes.

Copulation:

For successful copulation it is necessary that the male is able to stay on top of the female without being shaken off by unreceptive females. Moreover it must be able to trap the ovipositor of the female with his hind legs and direct it to his abdomen (Fletcher, 1989). Once the contact between female ovipositor and the tip of the male abdomen is established, copulation starts. The male uses his front legs to hold itself onto the wings or the thorax of the female. The males’ hind legs stroke the ovipositor of the female in the direction of its abdominal tip. I assume that this behaviour results in better and faster transfer of the males’ sperm. In several cases both sexes were seen to spread their wings during copulation. Stroking of the antennae of the female by the males with their own antennae was occasionally also observed. But there seemed to be no fixed patterns in these additional behaviours. During the copulation the female may walk along the thistle. Males and females are able to mate throughout their whole adult life (Fletcher, 1989; Fletcher & Prokopy, 1991; Zwölfer, 1974b).

3.1.1.2 Specific behaviour of the *U. cardui* females

Probing/Evaluation:

The female moves around the tip of an axillary bud. Here it examines the surface of the plant with its proboscis, which is tapped gently onto the plant surface several times. Examination of the surface is often followed by insertion of the ovipositor into the bud. The female seems to analyse the interior of the axillary bud (see chapter 7).

Probing can be distinguished from egg laying by the short duration of probing intervals and by searching movements of the female: it slowly swings from one side to the other, walks a few steps and then inserts its ovipositor into the same axillary bud again. The female may leave the bud for several seconds, but returns again. This evaluation behaviour can last a very long time, up to over an hour on a single bud, whereas a single probe of the bud always lasts less than ten minutes. Fig. 3.1 is a diagram of this behaviour.

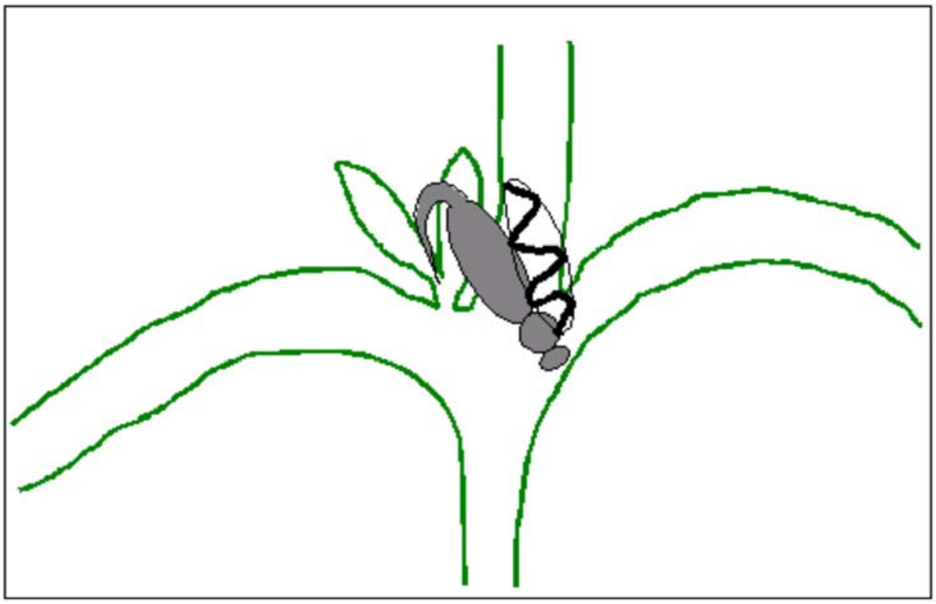


Fig. 3.1: Schematic drawing of the behavioural pattern probing. The ovipositor of the female is inserted into the axillary bud. The position of the female is the same in oviposition, but in these cases piercing of the bud lasts more than ten minutes and the female is resistant to disturbance.

Egg laying/Oviposition:

The *U. cardui* female pierces a bud with its ovipositor and stays for ten minutes or longer in this position. At this time it cannot be dislodged by disturbance from other flies, wind or similar factors. The female is absolutely motionless during oviposition. To be defined as egg laying in experiments it was essential that a gall developed later in that position, or that eggs were found upon dissection of the axillary bud, depending on the type of experiment. It was only necessary to revise egg laying to evaluation in two of 40 cases.

3.1.1.3 Specific behaviour of the males

Marking behaviour:

Usually an *U. cardui* male starts marking its territory at the tip of one of the larger apical leaves of the thistle. It marks the plant with a special secretion of the abdominal gland, composed of 4-methyl-3*Z*,5-hexadienoic acid (Frenzel *et al.*, 1990). The significant characteristic of this behaviour is thus the contact between the surface of the leaf and the tip of the abdomen. The male drags its abdomen across the surface of the leaves while walking. In most cases it rests after having marked one leaf; continuing after a short break of less than one minute (recorded as resting). Most of the males marked only one or a few of the upper leaves of the thistle and the stem, completely ignoring lower leaves. The marking behaviour does not seem to follow a certain scheme, not all leaves were marked, not all the marking–events took place consecutively and some males did not mark at all.

Courtship behaviour:

Encountering a female (or a male not immediately recognised as such) an *U. cardui* male performs a special courtship behaviour with the intention of copulating with the presumed female. This behaviour ensures the recognition of conspecific males by females (D. Stadler, unpublished), since the characteristic bands on the wings of the male are displayed to the female. The male spreads his wings, clearly presenting the banded marks on them (Zwölfer, 1974b). The male slowly moves from one side to the other and approaches the female. Near the female it starts gradually to circle the female with spread wings. This behaviour has been described in more detail for *U. cardui* and sister taxa by Zwölfer (1974b). If the female does not try to fly or walk away, the male tries to initiate copulation by mounting the female.

Threatening:

Threatening is performed only when meeting another male. Both males spread their wings, presenting each other the banded marks. The head and the thorax of the males are raised, their movements are faster than during courtship behaviour, resembling a kind of fast hopping dance. The males rock on the spot from the right to the left and wave their wings with the same rhythm as they move their legs. Often short threatening periods alternate with short flights or running on the plant. Usually the two confronting males fight afterwards. Threat behaviour often ended when males lost each other, mostly due to short flights to other plant parts. In these cases several minutes of very fast movement around the plant occurred, until the males seemed to calm down and started moving more slowly again.

Fighting:

Fighting was only rarely observed between females or a male and a female, but frequently in encounters between two males on the same plant. Usually there is at least a short threatening-period before fighting starts. The males then jumped at each other, grabbing the rival with their legs, banging their heads and vibrating their wings. The whole behavioural sequence is probably aimed at throwing the other combatant off the plant. This happened in approximately one third of the fights, in the last two thirds one of the opponents retreated. Often this led to a new cycle of threatening and fighting, since in not all cases did the retreating male leave the plant, or not even the leaf which formed the arena for the fight. In my observations fights with intermittent threatening periods lasted usually several hours. The longest fight observed lasted nearly five hours.

3.2 Material and Methods

The biology of the study system is elaborated in chapter 2.1.2.

3.2.1 Origin of plants and flies, general rearing

Thistles and rhizomes were collected in early spring, when the first leaves of the plants were visible. Different habitats were used for plant collection: a wet meadow, a large border of a crop field and the bank of a brook. Several preliminary experiments showed that the females did not discriminate between plants of different origin, thus the results of plants from different origins were pooled in all experiments.

Thistle growing and synchronization with emergence of the flies was performed with a similar breeding strategy as used by Sakuth (1996).

Plants were potted in soil (5 parts neutral humic soil, 2 parts pumice, 2 parts lava, 1 part loess, 2 parts peat and 2500g Osmokote^{TM} (a long-time fertilizer)/1m^3 soil)) and kept in the open to provide semi-natural conditions. In autumn and winter plants were grown in the greenhouse at L:D 15 (23°C):9 (18°C). The light conditions were achieved by an array of mercury lamps (Osram Power Star HQ I–TS 250 WINAL with a minimal light intensity of 400µEm^{-2}s^{-1}). The plants were watered twice a day to avoid drought stress, which would have affected female preference and larval performance. The thistles were repotted to larger pots after approximately two weeks growing and again two to four weeks later. The final pot size was three or five litres, according to plant size. Each plant was numbered, the height measured, and axillary buds and flower buds were counted before usage. The axillary buds were counted from the oldest leaves up to the top of the plant and numbered in the same direction. For multi-stemmed plants only the height of the largest shoot was recorded, but total shoot number was noted and all buds were counted as well. No thistle was used twice.

The *U. cardui* flies originated from several small populations within approximately twenty kilometres around Bayreuth, Germany. Some of the flies were part of the newly established laboratory population, although this experimental population could not be maintained without new flies from the field. The galls were collected in late summer and autumn and stored in the refrigerator until initiation of eclosure.

The flies emerged from these galls in Petri-dishes (ø 14 cm) and were then marked individually with a two colour-code (Hobbyline, Acryl - Colours in light green (10), blue (24), red (15), yellow (03) and orange (04)) on the wings. No interference of the marking with the behaviour was observed in preliminary comparisons. The same colour codes were used again if new individuals emerged from galls after the original possessor had died.

Between the experiments the flies were held in plastic cages (volume: 6l, Fauna box by Savic, Belgium) lined with regularly moistened cellulose tissue. They were fed *ad libitum* with 50% honey in water solution or sucrose-solution 67.5% (w/v) (Freese & Zwölfer, 1996; McCaffrey *et al.*, 1994). Water was also provided *ad libitum*. A maximum of 15 flies was kept per box. Two of the boxes were for single sexes, containing only males or females, to have a stock of virgin, naïve individuals. In most cases prior to experiments the flies were not confronted with thistles, in order to enhance individual motivation.

3.2.2 Behavioural experiments

The observation cages measured 0.5 x 1 x 0.5m (Fig. 3.2, Fig. 3.3). Observations were carried out in those cages established either inside a natural thistle patch or in an artificial thistle patch located on the roof of the university building. Inside the cage all naturally growing plants were removed and the two potted thistles were presented. Motivation of the flies was enhanced by natural light and climate condition and presence of more potential host plants. Observations started between 09:00–10:00 (CEST) in the morning and lasted until 15:00–16:00 (CEST) in the afternoon. Maximally six to eight individuals could be observed at one time while recording the behaviour of two of them in detail. Durations of the different behaviours were measured with an accuracy of one second with stop watches.

In three observations for each sex, data were recorded as movement pattern across the plant. These movement patterns were adapted to simplified thistle schemes in PowerPoint (PowerPoint 2000, Microsoft corporation). All data were organized in an Access database (Access 2000, Microsoft corporation). For ethogram-calculation the data of all individuals of one sex were pooled. The occurrence of one particular behaviour in all observations equals 100%. Accordingly the percentage of consecutive occurrence of the other behaviours was calculated. Transitions between the different behaviours were then organized graphically in PowerPoint. All further analyses and diagrams were carried out in SPSS 10.0 (SPSS Inc.).

To gain further information on the influence of host-plant suitability on behaviour, thistles were modified. Either form was changed by adding artificial leaves or all leaves of one thistle were sealed with nail polish to obscure any chemical and tactile information the flies may obtain. In these experiments the flies were positioned directly onto the modified host plant. The observations were also carried out inside a natural thistle patch to enhance the motivation of the flies. The same observations and analyses as on natural thistles were carried out.

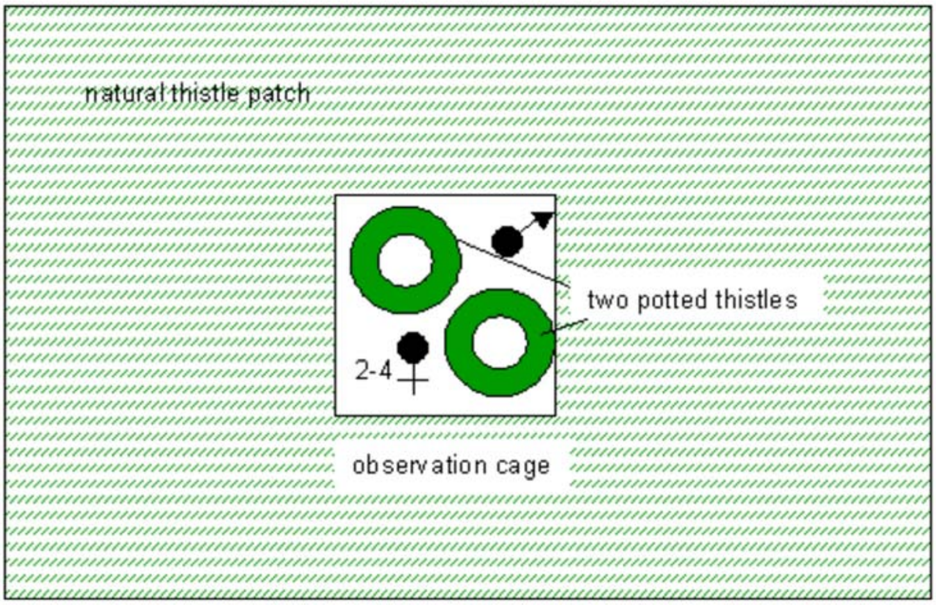


Fig. 3.2: Experimental design of behavioural observations. The observation cage was installed inside a natural (or an artificial) thistle patch. The same number of males and females (n=2-4) were observed on two host plants.

Fig. 3.3: The observation cage with *C. arvense* before addition of the flies.

3.3 Results

3.3.1 Behaviour on unmodified thistles

3.3.1.1 Females

In 66.3 observation hours 26 females were observed on natural host plants.

An example of the movement pattern of an individual female on a natural host plant is illustrated in Fig. 3.4. To simplify the figure and enable a comparison with the other movement-descriptions, the original observation was transferred to a schematic standard thistle, which underestimates the natural number of leaves. Action patterns were preserved. In order to show the main characteristic patterns, but not to present too many confusing detailed movements, one of the shorter observations was chosen to demonstrate the typical movement pattern of a female on a natural host plant.

The preferred resting and grooming places were at the top of the plant. The top of the host plant was also the place where most of the probing behaviour and potential egg laying occurred. This may be due to the fact that the first axillary buds develop at the bases of the top leaves of the plant and a female is thus most likely to find suitable buds for oviposition at the top of the host plant.

To give a better overview of the behavioural sequence the observation of the same female is additionally presented as a flow chart in Fig. 3.5.

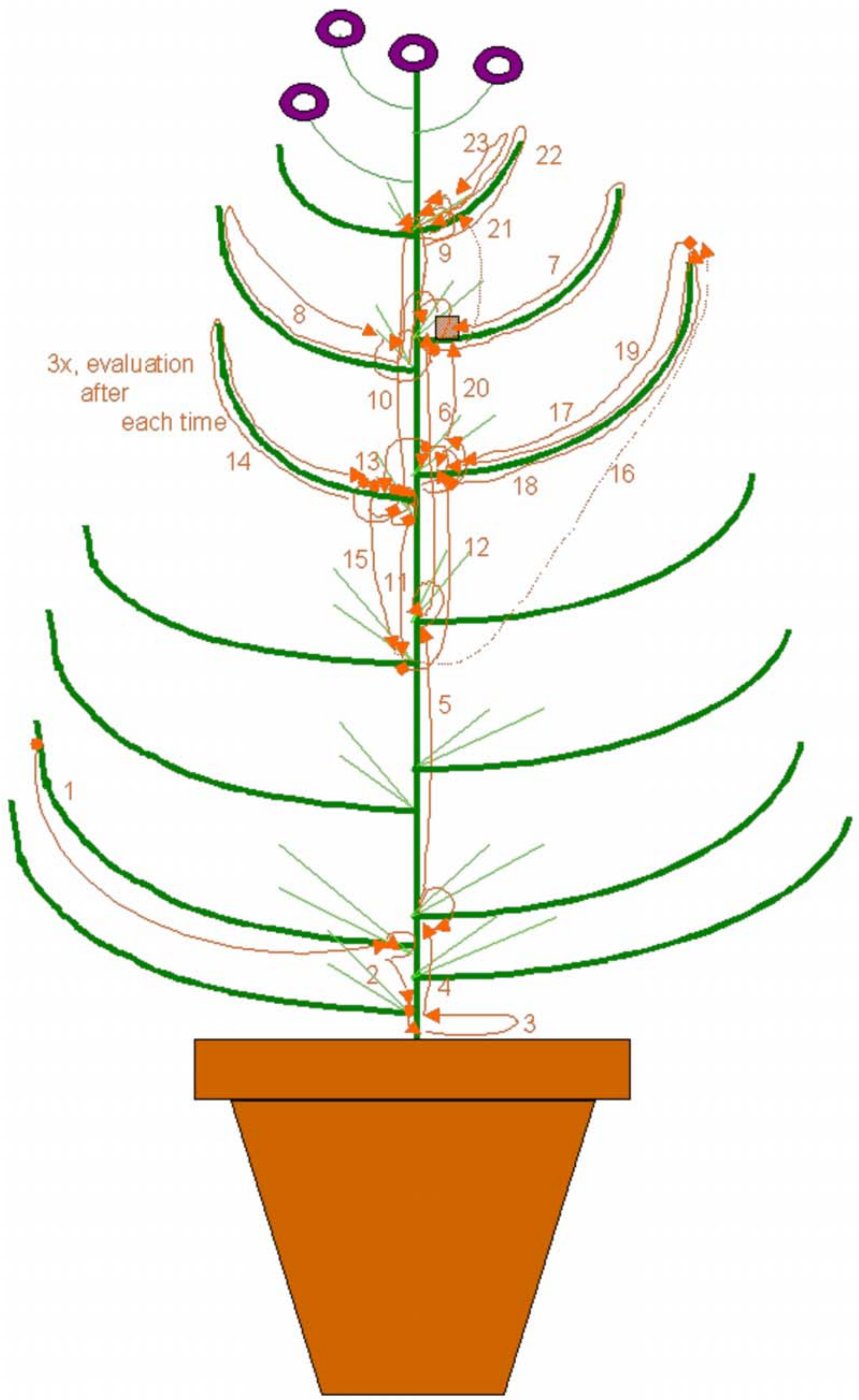


Fig. 3.4: Movement pattern of a female on a natural host plant. Duration of this observation: 121.7 min. Numbers describe the order of movement around the plant. Solid line: movement by walking, dotted line: flight, circle: resting, rhombus: grooming, circuiting arrows: evaluation, square: egg laying.

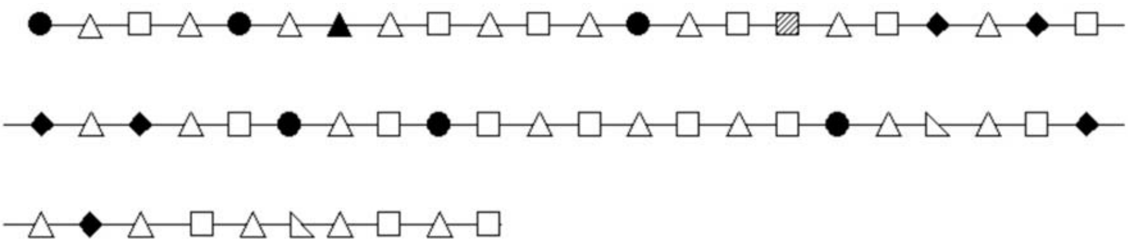


Fig. 3.5: Flow diagram of the movement pattern of a female on a natural host plant. Duration of the observation: 121.7 min.

● = resting
△ = running on the plant
☐ = evaluation
▲ = running
▨ = egg-laying
◺ = flying
◆ = grooming

The observations of 26 females were pooled to calculate an ethogram and present a more general behavioural sequence (Fig. 3.6). Since the full ethogram was very complex it was simplified, showing only behaviour-transitions higher than 10%.

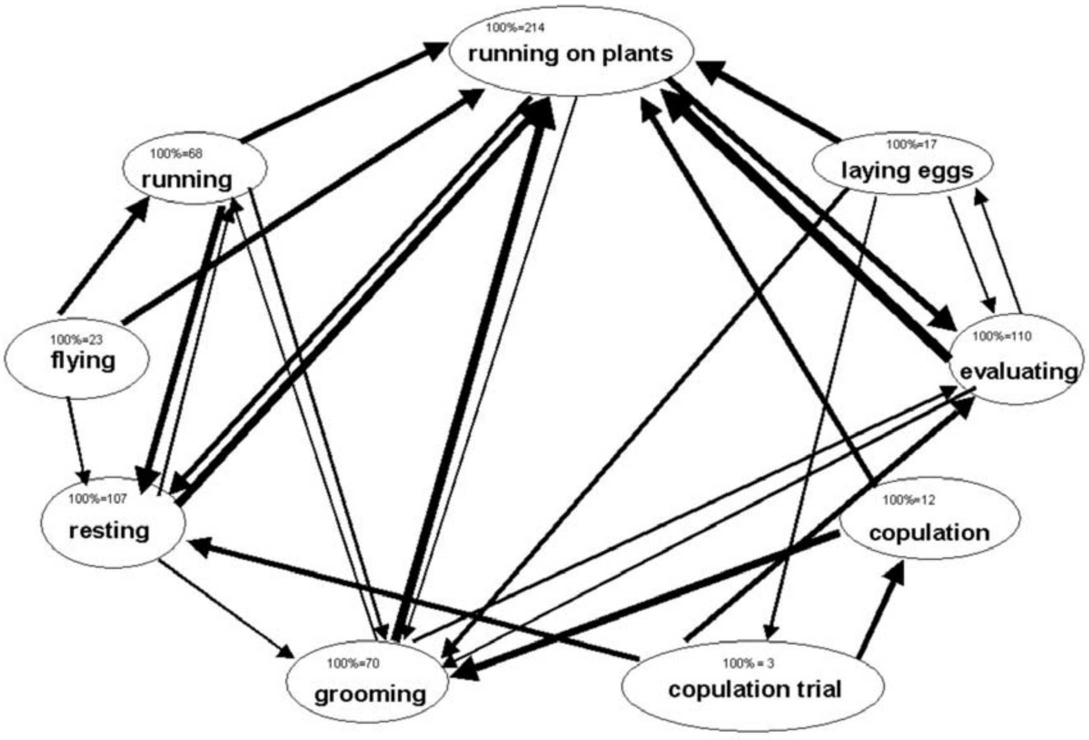
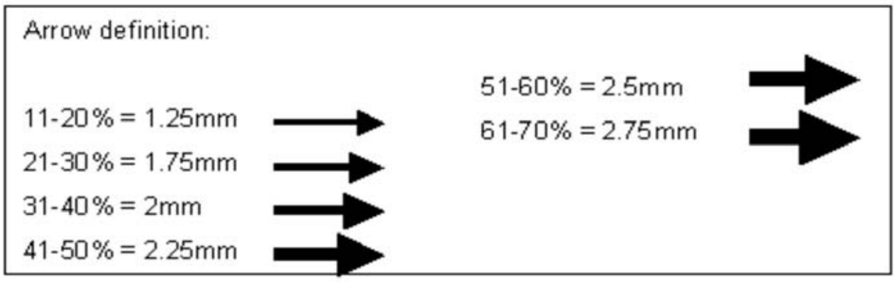


Fig. 3.6: Ethogram of behavioural observations of 26 females. The arrow-width represents the percentage of transitions to other behaviours:



Arrow definition:
11-20% = 1.25mm
21-30% = 1.75mm
31-40% = 2mm
41-50% = 2.25mm
51-60% = 2.5mm
61-70% = 2.75mm

It is interesting that only evaluation behaviour preceded egg laying. Running on plants, grooming, resting and evaluation were all closely linked to each other. However, despite the connection between evaluation and egg laying, there were no other obviously fixed behavioural loops. Running was in 43% of cases followed by resting. In 32% of cases it was followed by running on the plant (in these cases one of the leaves of the plant touched the netting of the observation cage). Flying was in 35% of cases followed by running on the plant and in 39% by running. Running on the plant occurred after most other behaviours. In 31% of cases it was followed by resting and in 41% by evaluation. All other transitions were of minor importance. Resting was most often followed by a continuation of the movement around the plant (59%). Grooming events seem to have been a short break in the movement of the flies around the plant (54%). Copulation was followed either by running on the plant (33%) or by a grooming period (42%). Although evaluation was the only behaviour that led to egg laying, in 63% it was followed by running on the plant again, and in only 16% of cases females oviposited. Transitions into running on the plant signify a long-lasting decision-behaviour of the females until they finally decided on a particular axillary bud in which to oviposit. Egg laying led to movement around the host plant in 41% of cases. No influence between the age of the respective female and its oviposition performance could be observed.

The time the females spent on the different behaviours was measured. If the time required for particular behaviours was summed for each of the individual females, high inter-individual variability became apparent (Fig. 3.7). Not all of the females were observed probing axillary buds and ovipositing. Copulations were also not observed for every female. Time spent on running, resting, evaluation and egg laying showed extremely high differences between individuals. In contrast time spent on grooming and running on the plant seemed to consume a similar amount of time.

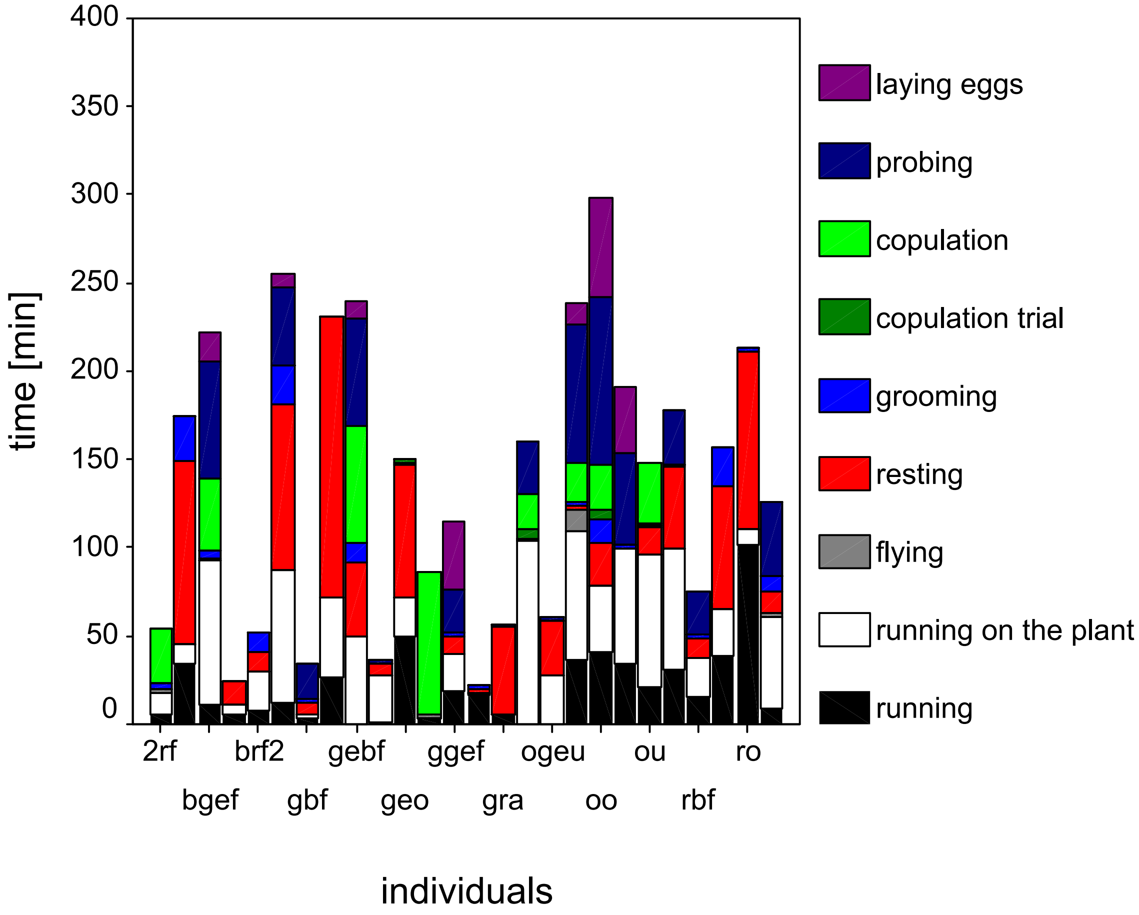


Fig. 3.7: Time budget of females observed on host plants. Y-axis: sum of the time spent [min], x-axis: individual females, each represented in one column.

For analysis of duration of the different behaviours performed by females the mean durations of each particular behaviour were calculated. The proportion contributed by these particular behaviours to total duration is represented in Fig. 3.8. Running, running on the plant, copulation and resting required most of the time budget of females.

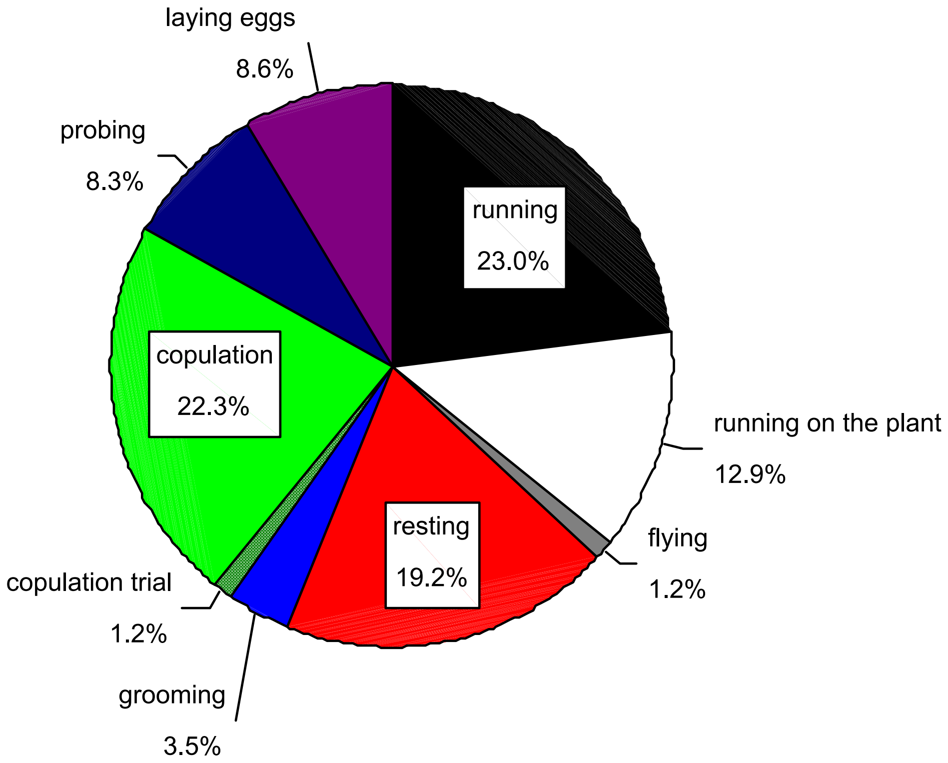


Fig. 3.8: Mean percentage of the time spent on different categories of behaviour by females (n=26) observed on natural host plants.

3.3.1.2 Males

In 48 hours observation four males were observed in detail.

An example of the movement pattern of a male is shown in Fig. 3.9. Like the female, the male moved around the whole thistle. Unlike the female, it walked many times up and down the plant. Especially during marking events the movement was quite extensive. Resting and grooming was performed in between on different parts of the plant.

The behavioural sequence of the same observation was depicted additionally as a flow chart (Fig. 3.10) to give a better overview of the behavioural flow on a host plant.

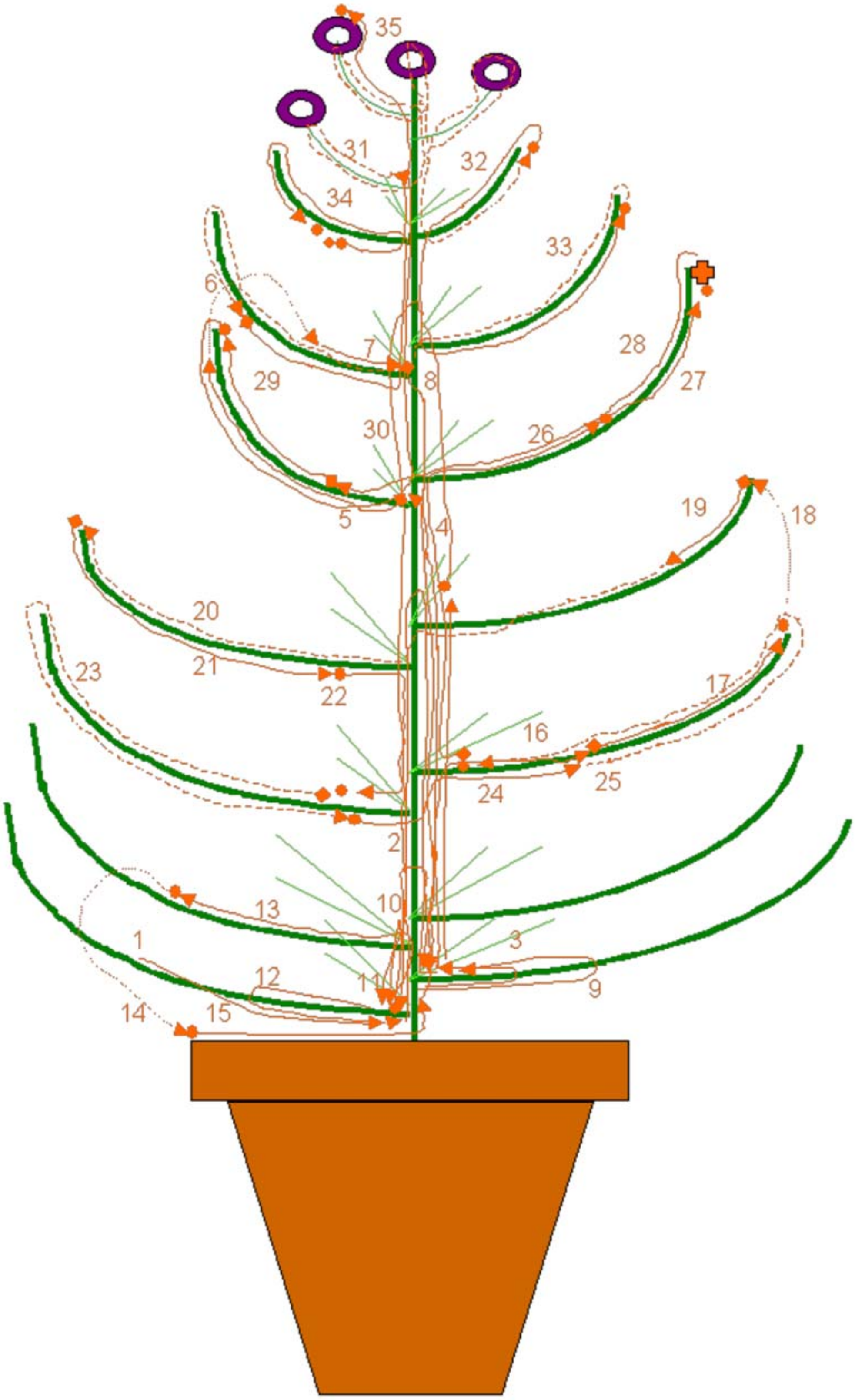


Fig. 3.9: Movement pattern of a male on a natural host plant. Duration of this observation: 167.9 min. Numbers describe the order of movement around the plant. Solid line: movement by walking, dotted line: flight, dashed line: marking behaviour, circle: resting, rhombus: grooming, cross: copulation.

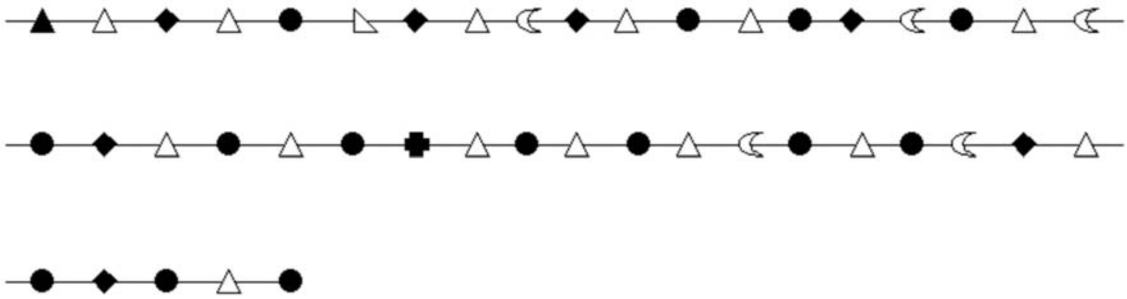


Fig. 3.10: Flow diagram of the movement pattern of a male on a natural plant. Duration of the observation: 167.9 min.

● = resting
△ = running on the plant
☾ = marking
▲ = running
◺ = flying
✚ = copulation
◆ = grooming

If the observations of the 4 males, which were observed in detail are combined it was possible to calculate an ethogram (Fig. 3.11), albeit with smaller database than for female behaviour. In order to simplify the figure only behaviour-transitions above 10% were included.

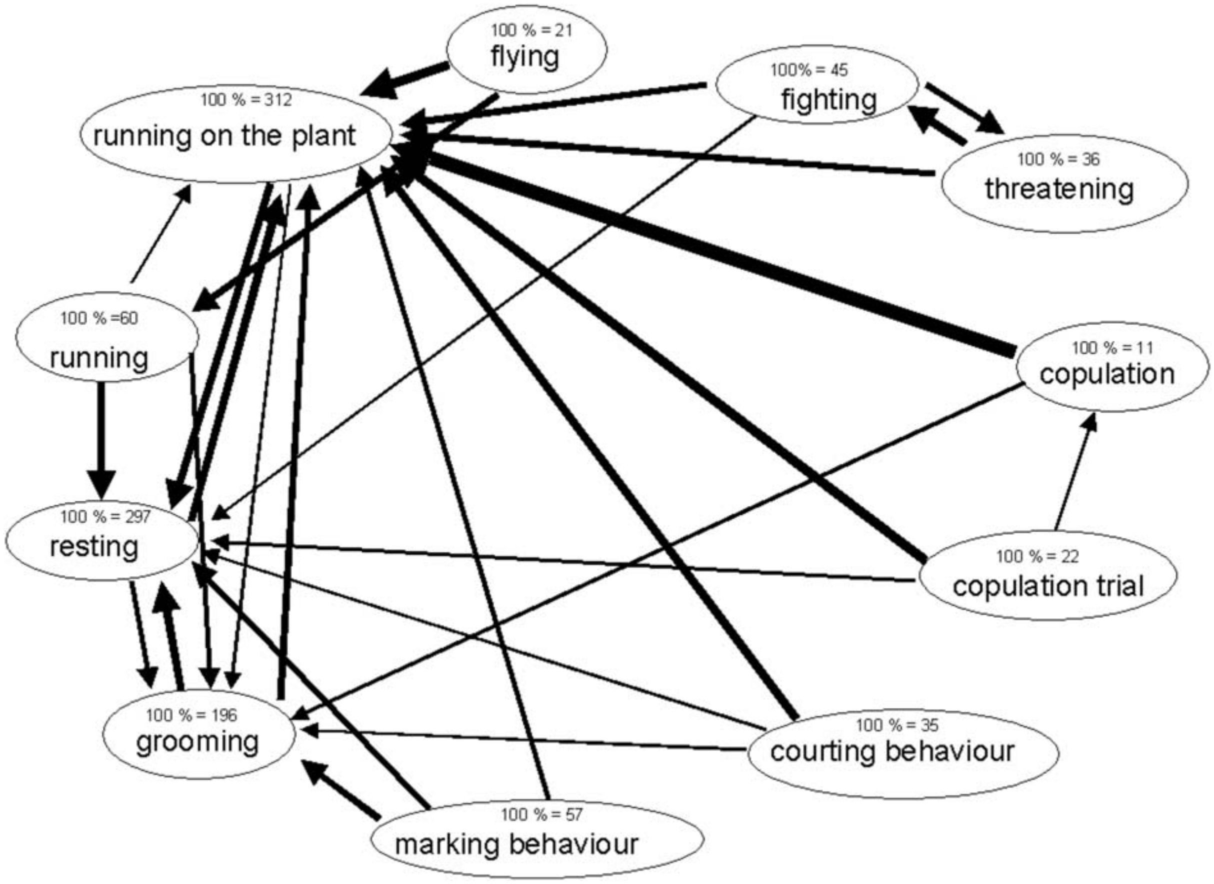


Fig. 3.11: Ethogram of behavioural observations of 4 males. The arrow-width represents the percentage of transitions to other behaviours:

Arrow definition:

11-20% = 1.25mm →
21-30% = 1.75mm →
31-40% = 2mm →
41-50% = 2.25mm →
51-60% = 2.5mm →
61-70% = 2.75mm →
71-80% = 3mm →

In 22% of cases running was followed by running on the plant, signifying that the male was able to step from the walls of the observation cage directly onto the host plant. In 25% of cases after running it groomed itself. However, more often (50%) running was followed by resting. Flying was followed by running on the plant (52%) or by running (33%). After running on the plant all other behaviours followed, the most prominent ones were grooming (18%) and resting (46%). Resting interrupted nearly all other behaviours, especially running on the plant, grooming and running, and these were recommenced after resting (running on the plant 43%, and grooming 30%). Grooming played a similar role, with transitions to running on the plant (38%) and resting (45%) occurring most often. Copulation attempts were followed by copulation in only 18% of cases, more often they were followed by resting (13%) and running on the plant (59%). This shows, that most of the females avoided rape by reacting in nearly all cases with fleeing. Successful copulation was followed by running on the plant (72%), or in several cases (18%) by grooming. Marking behaviour was interrupted either by running on the plant (25%), by resting (37%) or by grooming (39%). Courting was succeeded most often not by a copulation (2.9%) or copulation trial (8.6%), but by running on the plant again (49%); in 20% of times it was interrupted by resting and in 11% by grooming. Threatening was almost exclusively linked to both running on the plant (38%) and fighting (50%). In 38% of cases, fighting was followed by running on the plant, signifying that one of the opponents retreated, or that they lost one another during the fight. In 11% of cases the fight was interrupted by resting. In a further 33% another threatening period took place. In addition to the close linkage of all different behavioural aspects to running on the plant, there exist close relations between threatening and fighting, between copulation trial and copulation, between marking behaviour and grooming, between marking and resting and between resting and grooming.

As with the females, high inter-individual variability was observed in the males (Fig. 3.12). The time spent on resting and grooming was quite similar in all males, but time spent on running on the plant, copulation, threatening, marking and courting behaviour was different.

Copulation was the behaviour with the longest duration (Fig. 3.13). Among the others resting, running and running on the plant occurred often for longer periods. It is noteworthy that the time spent on most of the behaviours is more evenly distributed than in the case of the females. The only exception is the time the males spent on copulation.

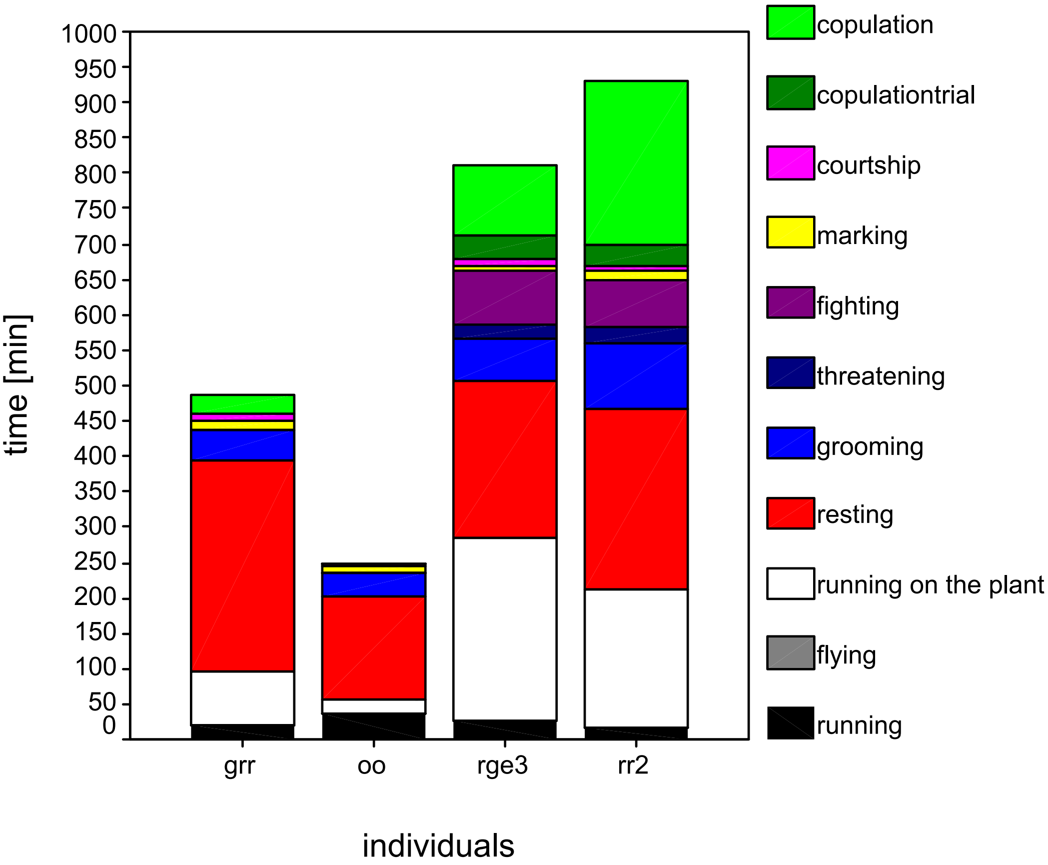


Fig. 3.12: Time budget of four males observed on natural host plants. Y-axis: sum of the time spent [min], x-axis: individual males, each represented by one column.

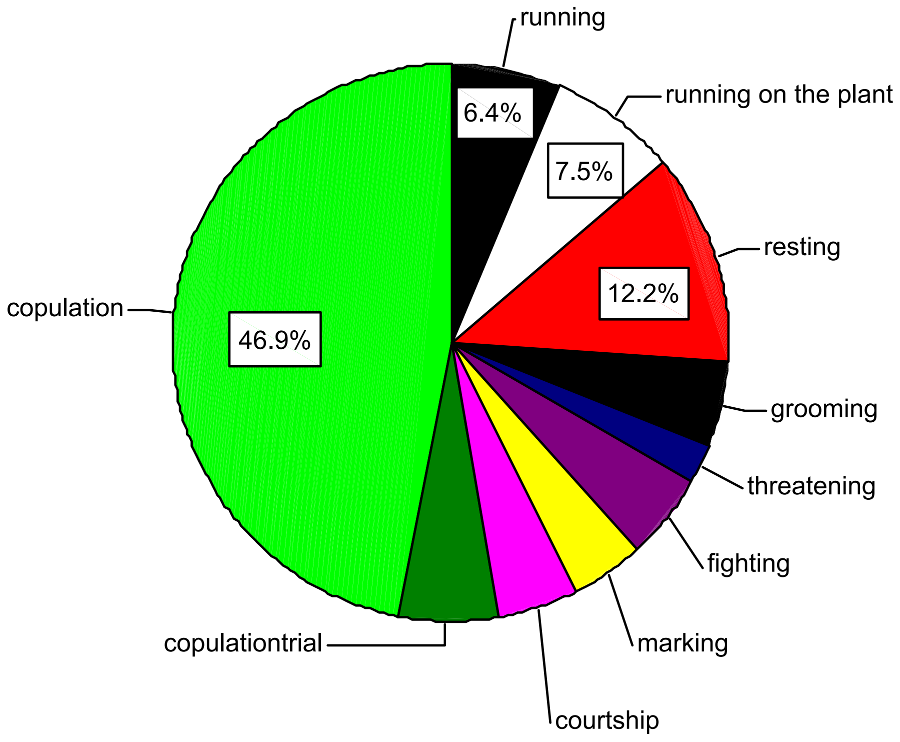


Fig. 3.13: Mean percentage of the time spent on different categories of behaviour type by males (n=4) observed on natural host plants.

3.3.2 Behaviour on modified plants

To render host plants completely unsuitable for oviposition, plants were transformed artificially. It was investigated, whether the flies exhibited disturbed behaviour on a modified host plant (no-choice situation), the degree of possible disturbance and which behavioural categories were predominantly affected.

3.3.2.1 Females

If female flies encountered host plants bearing additional plastic leaves the fly behaved the same as on a natural host plant until it encountered the first of the artificial leaves. All 16 females, which were tested flew off immediately and did not return for the remainder of the experiment.

Of the ten flies, which were tested on nail polish sealed plants only two remained long enough on the plant to enable thorough observation. The remaining eight females of *U. cardui* left the plant in less than thirty minutes. The two females, which stayed on the plant longer were observed for 1.7 hours in total.

In Fig. 3.14 an example of the movement pattern of a female on a host plant with nail polish on the leaves is shown. By comparison with Fig. 3.4, it is obvious that the female did not move around the whole plant, it exhibited a restricted movement without oviposition and a reduced amount of evaluation behaviour. In particular running on the plant was restricted to even smaller areas than was generally observable in female flies.

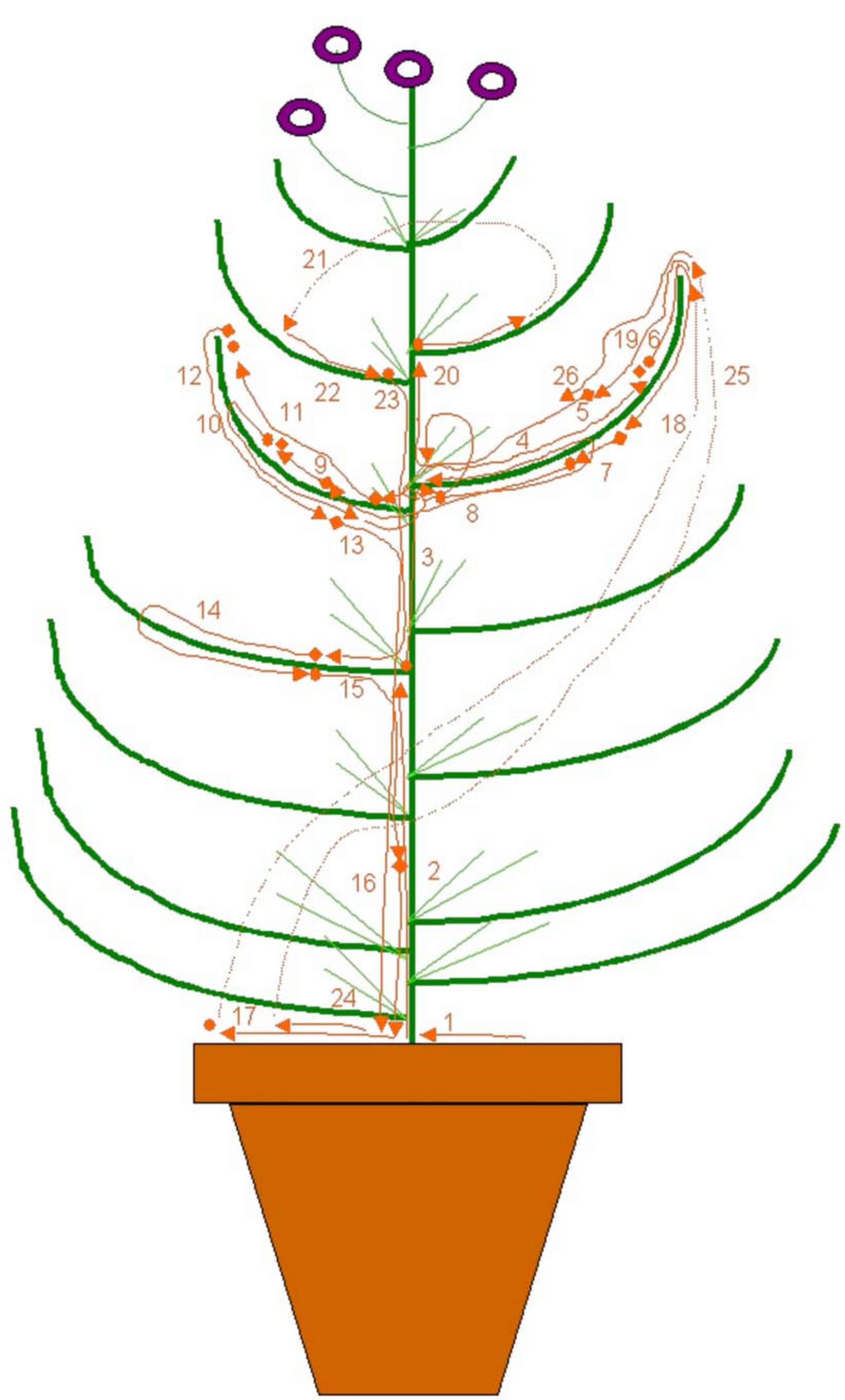


Fig. 3.14: Movement pattern of a female on a plant sealed with nail polish. Duration of the observation: 59 min. Numbers describe the order of movement around the plant. Solid line: movement by walking, dotted line: flight, circle: resting, rhombus: grooming, circuiting arrows: evaluation.

To provide a better overview of the behavioural sequence the same observation is shown in Fig. 3.15 as a flow chart. In comparison with Fig. 3.5 it is obvious that there was less evaluation. And, although the observation time was shorter, there occurred more grooming. Furthermore very short grooming events (less than five seconds and therefore not recorded), interrupting running on the plant were performed in high frequency. The enhanced grooming-activity cannot be ascribed to sticking of the flies to the polish, since it had dried completely. For a human nose it did not smell any more also, but it is possible, that the nail polish odour disturbed the female flies.

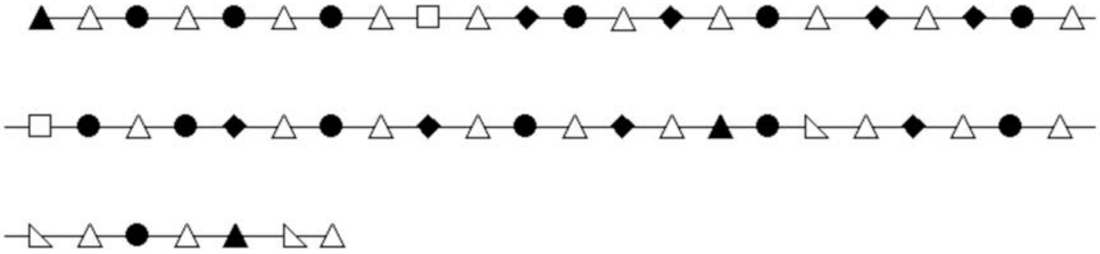


Fig. 3.15: Flow diagram of the movement pattern of a female on a plant sealed with nail polish. Duration of the observation: 59 min.

● = resting
▲ = running
△ = running on the plant
□ = evaluation
◺ = flying
◆ = grooming

Although the database was much smaller than in the experiments on natural host plants, an ethogram of the behaviour of the two females was calculated. There existed fewer transitions between behaviours (Fig. 3.16), due to the fact, that the females did not perform all behaviours. Only running on the plants, running, flying, resting, grooming and evaluation occurred.

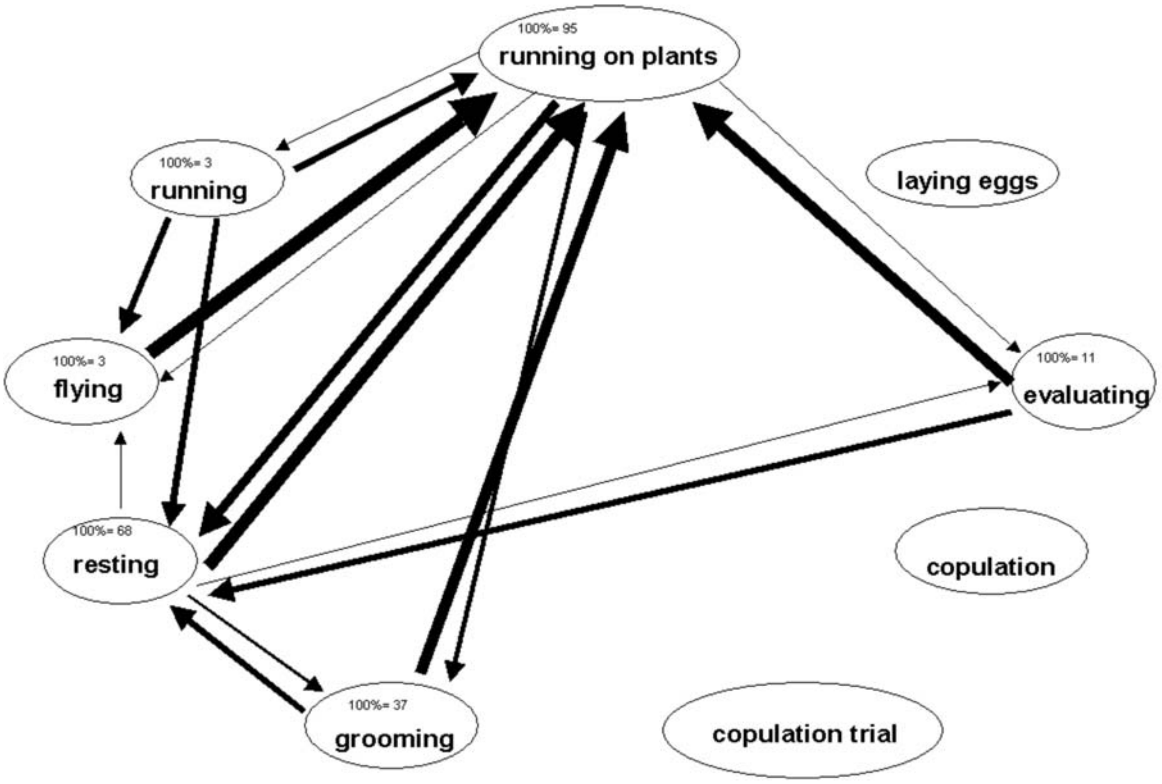
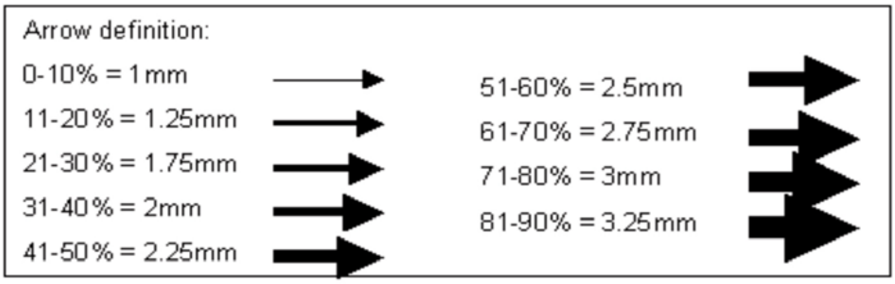


Fig. 3.16: Ethogram of the behaviour of two females performed on plants treated with nail polish. The arrow-width represents the percentage of transitions to other behaviours:



Arrow definition:
0-10% = 1mm
11-20% = 1.25mm
21-30% = 1.75mm
31-40% = 2mm
41-50% = 2.25mm
51-60% = 2.5mm
61-70% = 2.75mm
71-80% = 3mm
81-90% = 3.25mm

The behaviour running occurred only three times, once it was followed by running on the plant, once by resting and once by flying. Flying was only observed three times; all these times it was followed by running on the plant. In 29% of cases the behaviour running on the plant was performed, it was followed by grooming, in 57% by resting. Resting was observed interrupting other behaviours, as in the behaviour of females on natural thistles. Transitions occurred to running on the plant (79%), grooming (18%), flying (1%) and evaluation (1%). Grooming interrupted running on the plant even more, than in observations of females on natural host plants (68%). In 32% of cases grooming was followed by resting. 64% of the evaluation-events again led to running on the plant. Despite the enhanced occurrence of grooming the main characteristics of the behaviour transitions remained on host plants modified with nail polish.

The behaviour of the two females on the modified plants was quite similar (Fig. 3.17). Both females performed the same behaviours (except running, which was only performed by one of the two females) for nearly the same time. The average time the females spent on each behavioural category is presented in Fig. 3.18. Running on the plant, resting, or grooming have the longest duration. If the results are compared to the behaviour on natural host plants it is obvious, that the females need more time for grooming and they groom for longer periods on plants modified with nail polish. The duration of the other behaviours is in the same range.

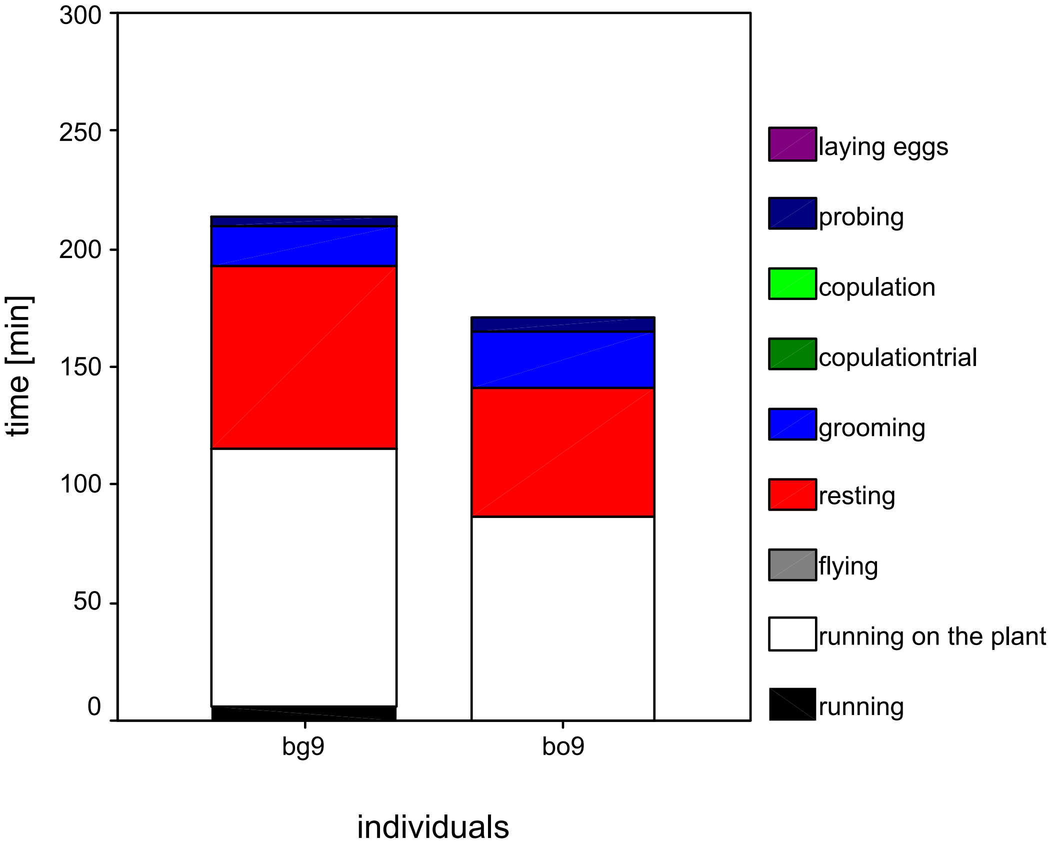


Fig. 3.17: Time budget of females observed on modified host plants. Y-axis: sum of the time spent [min], x-axis: individual females, each represented by one column.

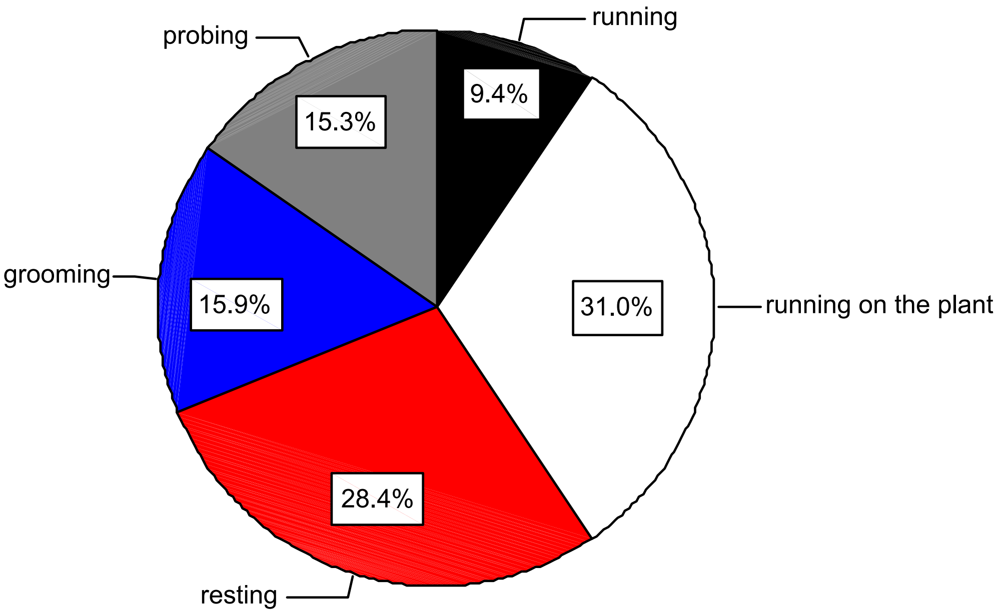


Fig. 3.18: Mean percentage of the time spent on different categories of behaviour by females (n=2) observed on modified host plants.

3.3.2.2 Males

Twelve males were tested on plants with additional artificial plastic leaves. Nine of the males left their host plant immediately after the encounter of the first of the artificial leaves. The three other males continued to behave in the same way as on a natural host plant.

On plants sealed with nail-polish ten different males were tested, but only two of them remained longer than half an hour on the plant. Only 2.6 observation hours, for these two males, were recorded.

The movement of a male on a modified plant was restricted to small parts of the plant (Fig. 3.19). Movement around the whole plant was rare; extensive up and down movement (see Fig. 3.9) did not occur. Marking behaviour was also rare.

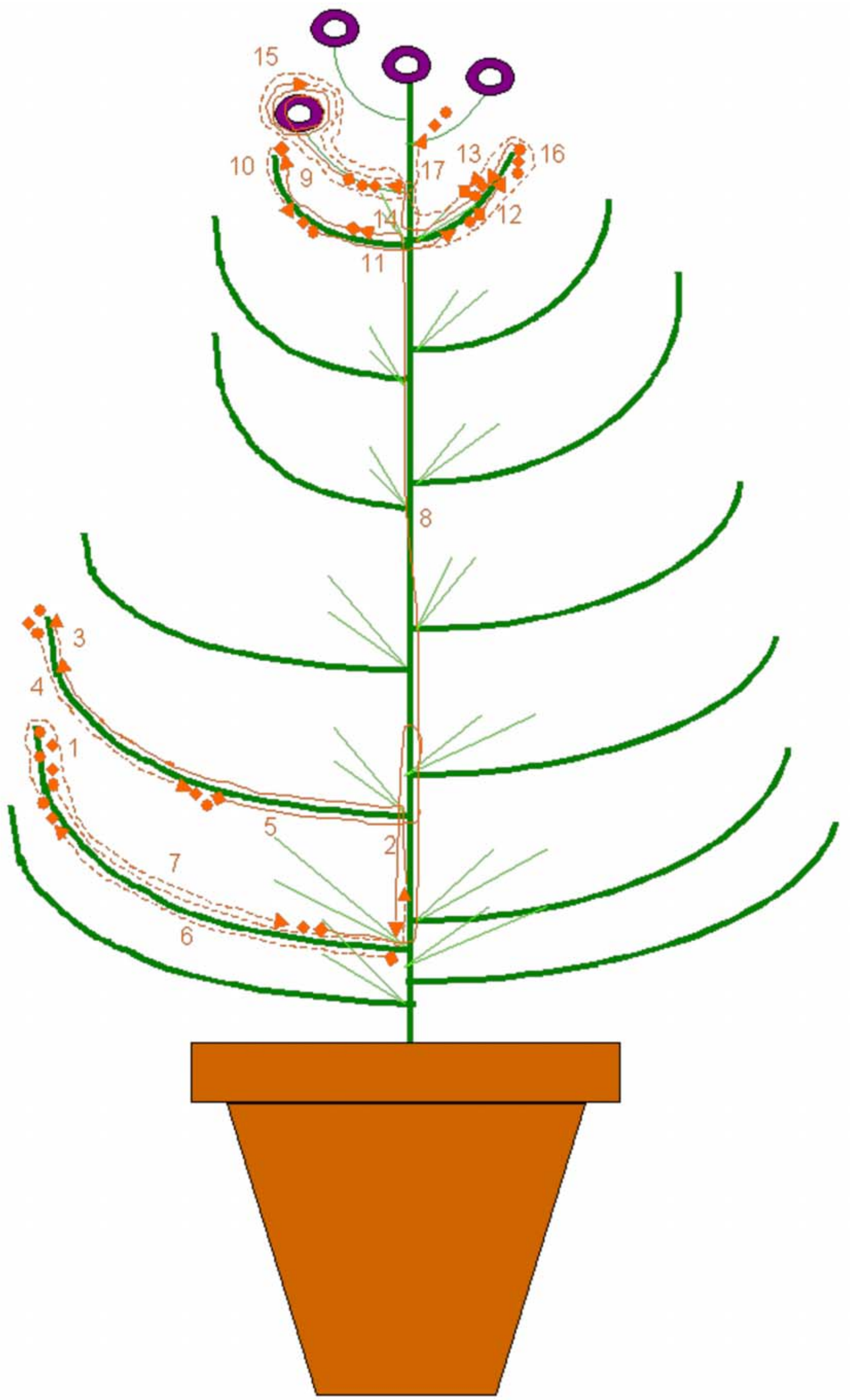


Fig. 3.19: Movement pattern of a male on a plant sealed with nail polish. Duration of the observation: 83 min. Numbers describe the order of movement around the plant. Solid line: movement by walking, dotted line: flight, dashed line: marking behaviour, circle: resting, rhombus: grooming.

Additionally the same observation was depicted as a flow chart (Fig. 3.20). This presentation shows evidently, that the male groomed itself more often than on a natural host plant (Fig. 3.10). It is the most prominent difference, if only the sequence of the behaviour is analysed.

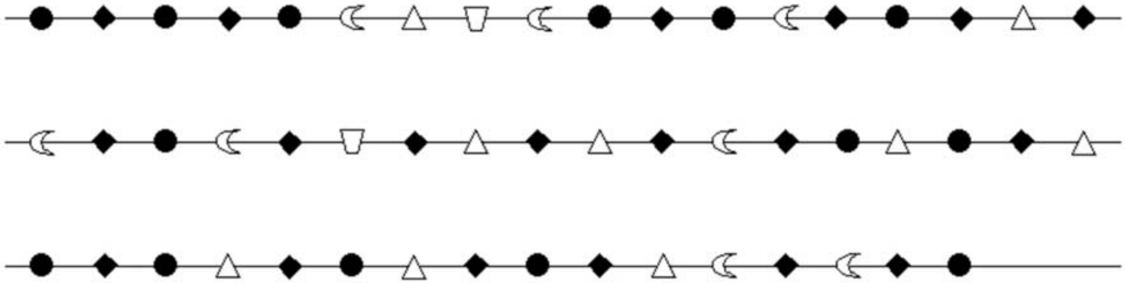


Fig. 3.20: Flow diagram of the movement pattern of a male on a plant sealed with nail polish. Duration of the observation: 83 min.

● = resting
△ = running on the plant
▽ = threatening
☾ = marking
◆ = grooming

Ethograms of male behaviour were calculated, although fewer males were tested than on natural host plants. Only running on the plant, resting, grooming, marking and threatening occurred as behaviours (Fig. 3.21).

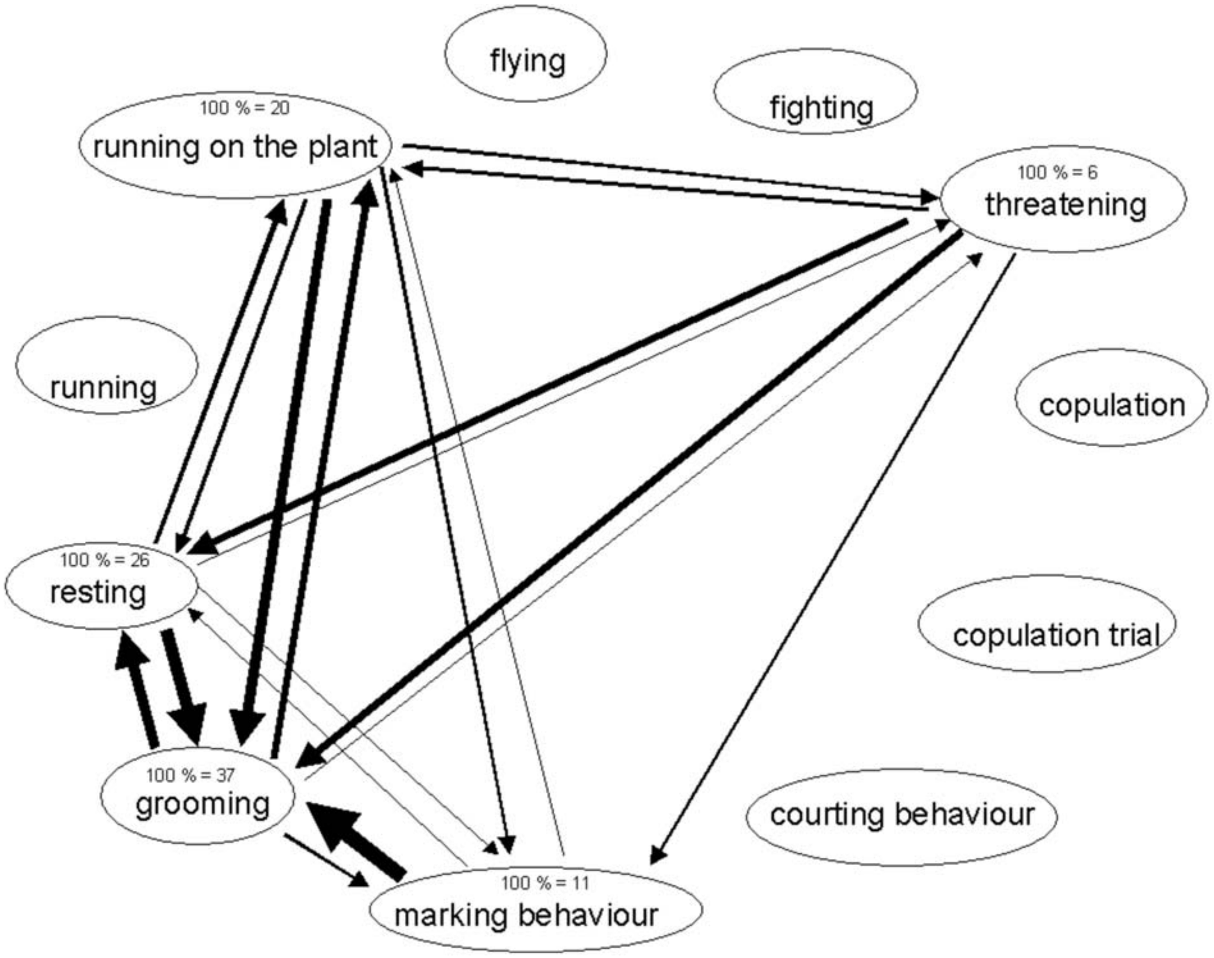
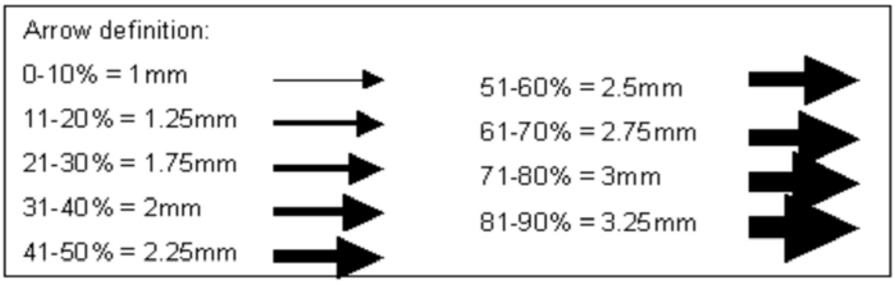


Fig. 3.21: Ethogram of the observations of two males on host plants sealed with nail polish. The arrow-width represents the percentage of transitions to other behaviours:



Running on the plant was followed either by marking (15%), resting (15%), threatening (15%) or by grooming (55%). Resting and grooming again often interrupted other behaviours, especially running on the plant. Resting led to running on the plant again in 23% of cases; in 65% of cases to grooming. Grooming was followed by marking occasionally (11%), but most often by running on the plant (32%) or resting (54%). In 83% of cases marking was followed by grooming, this relationship was more prominent than on natural host plants. Threatening occurred only six times. In one case it was followed by running on the plant, or marking; in two cases each it was followed by grooming and resting.

If the time the males spent on the different behaviours on host plants sealed with nail-polish was calculated (Fig. 3.22), the same result as for the females is obtained: treatment with nail-polish seemed to diminish individual variability. But it is noteworthy, that in both cases n=2 only. Fig. 3.23 shows the mean duration of the different behaviours performed on modified host plants. If the time spent on the particular behavioural events is compared with the time spent on natural host plants there are more differences as with females. Especially resting, grooming and running on the plant require more time per one event. However, the males did not copulate on modified host plants, a behaviour which had the longest duration on natural thistles.

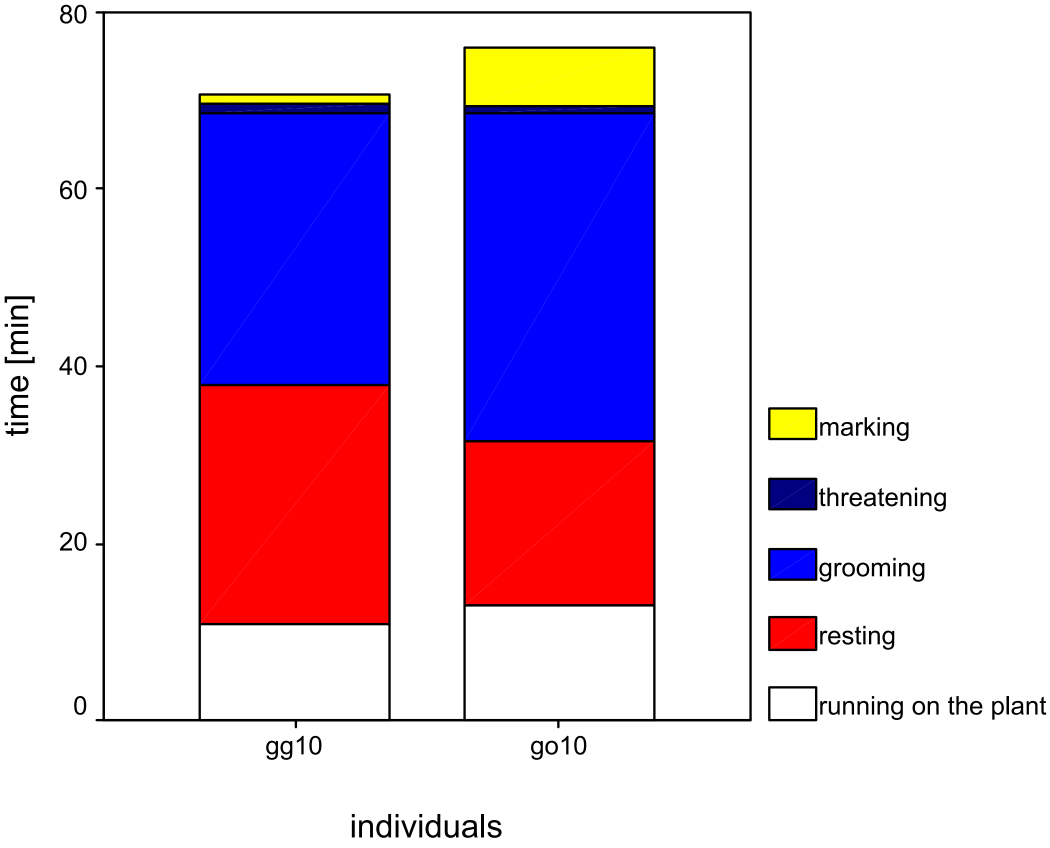


Fig. 3.22: Time budget of two males observed on modified host plants. Y-axis: sum of the time spent [min], x-axis individual males, each represented by one column.

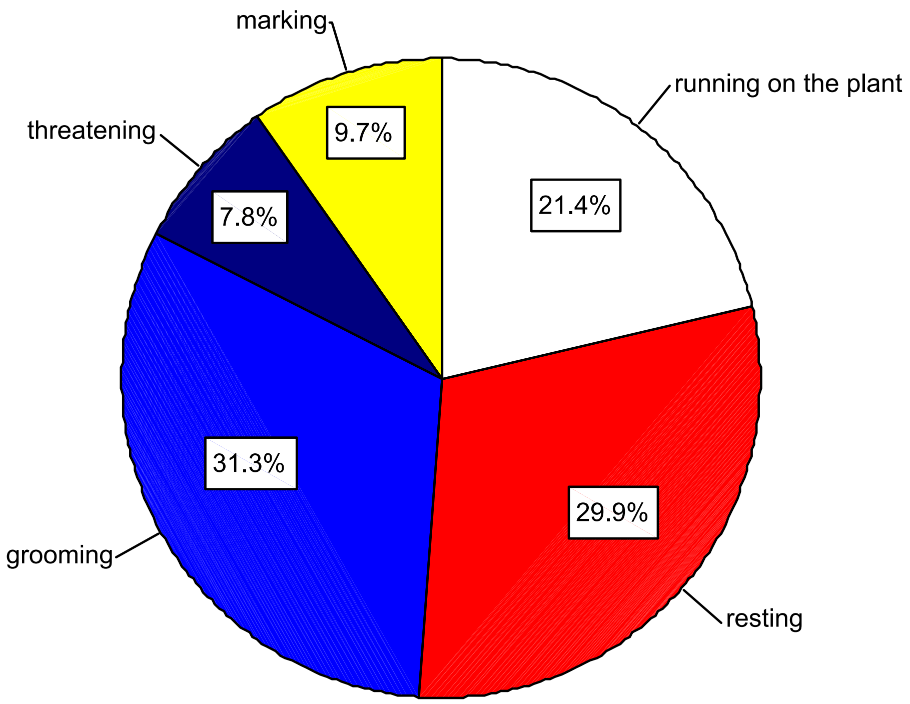


Fig. 3.23: Mean percentage of the time spent on different categories of behaviour by males (n=2) observed on modified host plants.

3.4 Discussion

On natural host plants, *U. cardui* females tended to restrict their movement to the upper part of the plant, where it is more likely that they will encounter suitable axillary buds for oviposition. The females seldom walked long distances on the plant. If the plant was modified with nail polish sealing the movement of the females was even more restricted to small areas of the plant. If females encountered additional artificial leaves they took off immediately and did not return to the plant, which indicates that they no longer distinguished it as a suitable host plant. The same applied for most of the females and males observed on plants sealed with nail polish. The two females and the two males, which were observed for a longer period were probably individuals, which had less rigid host plant templates. The fact, that most individuals avoided modified host plants indicates the relative rigidity of the host plant template employed in the foraging behaviour. Unfortunately, no comparable studies have been published for other tephritids.

If the movement pattern of the male and the female was compared, it is obvious that females tended to move less up and down the plants. Their movement was more restricted to small areas, in contrast to the movement of the males, which used the whole plant. These differences between male and female movement patterns remained constant in all observations performed on natural host plants. Even flies, which were not motivated to mark their host plant or lay eggs showed these characteristics in their movement pattern. On the plants, which were modified with nail polish the behavioural change in the males was more evident than for the females. Movements between the upper and the lower parts of the plant took no longer place. Running on the plant was restricted to very small areas, as was marking. Like the females, the males showed enhanced grooming activity, a relationship between marking, grooming and resting was more prominent than on natural host plants. These aspects, observed on the individuals, which stayed longest on the plants, were also observed for the other individuals before they left the modified thistle. Again no comparable studies have been published.

If the time spent on different behaviours was compared between single females or males the most noticeable result was high individual variability. Comparison between the behaviour on natural and modified host plants should be considered with caution, since individual variability may have influenced the outcome of these experiments. Although the nail polish seemed to diminish individual variability, it is still possible that different individuals may react differently to sealing with nail polish.

Especially in the case of weed- and pest management, individual variability in behaviour concerning host selection might be a problem, as long as the determinants of the variability are not known (Roitberg, 1990). Individual variability in insect behaviour leads to higher unpredictability: however pest management requires a certain amount of predictability to ensure constant success of management efforts (Ananthakrishnan, 1999; Mattiacci *et al.*, 1999; Roitberg, 1990; Schmidt, 1994). Thus, the high variability among *U. cardui* females may be one of the reasons, why its success as a control agent against *C. arvense* in Canada has been low (Peschken *et al.*, 1982).

Males of *U. cardui* require most of the time spent on the larval host plant for copulation. The behaviours performed by the males, especially threatening and courting, showed the importance of the wing pattern. Wing displays like the ones described here for the males of *U. cardui*, occur in nearly all other fruit fly species with banded wings (Headrick & Goeden, 1999; Zwölfer, 1974b). The wing displays offer the opportunity to relate morphological characters and associated behaviours in an evolutionary context. The banded wings and the associated wing displays help the closely related fruit fly species to discriminate conspecifics from individuals of other species (sexual selection) (Zwölfer, 1974a; Zwölfer, 1974b). Since many fruit fly species in Europe use *Carduus* and *Cirsium* species as larval hosts, which usually occur together in the same habitats, recognition of a conspecific mating partner is quite important (Headrick & Goeden, 1998). The wing displays are the main communication patterns members of the same and opponent sex (Zwölfer, 1974b). Males use communication by wing displays to mark and defend resource-based territories, which serve as a “rendezvous-place” (Zwölfer, 1974a), while communication between males and females ensures mating. Copulations attempts of males on unreceptive or unwilling females from behind were usually unsuccessful. The same result has also been observed in several other fruit fly species of the subfamily Tephritinae (Headrick & Goeden, 1999), which further highlights the importance of wing displays preceding copulation. A secondary function of these displays may be mimicking the greeting dance of salticid spiders, which are the main predators of the adults (Headrick & Goeden, 1999). The spiders may then mistake the fly as a conspecific.

The observed females of U. *cardui* spent less time on copulation than the observed males. Most time is required for running, running on the plant and resting. As shown in the ethogram running on the plant is linked to probing behaviour. Concerning the host plant selection behaviour of female phytophages, it is generally assumed that they are able to assess the quality of a host plant stand (Charnov, 1976; Pyke, 1984). Encountering an individual host plant, they estimate its quality and add the assessments of several hosts to an image of the overall host quality in a host plant stand (Roitberg, 1990). The quality of potential host plants is detected mainly with the ovipositor (Austin & Field, 1997; Casiraghi *et al.*, 2001; Chadha & Roome, 1980; Diaz-Fleischer *et al.*, 1999). Similar probing into host plant or host tissue with the ovipositor are reported from a variety of herbivorous or parasitoid insects (Kopelke, 1998; Sanders, 1962) as well as fruit flies (see for example Casiraghi *et al.*, 2001; Chabi-Olaye *et al.*, 2001; Chadha & Roome, 1980; Diaz-Fleischer *et al.*, 1999). Here probing of the host (plant) tissue is often accompanied with antennal drumming and vibration (Chadha & Roome, 1980; Schmidt, 1994). In the case of the *U. cardui* females this behaviour was not prominent, the ovipositor seems to be more important in the quality assessment of the host plant. Another behaviour, which was never observed in *U. cardui* or in any other *Urophora* species was marking of the host with a deterrent pheromone (Hoffmeister & Gienapp, 1999). This has been reported from many other tephritid genera, where it serves to diminish the risk of additional oviposition by conspecific females (intra-specific parasitism) (Diaz-Fleischer *et al.*, 1999; Lalonde & Roitberg, 1992). Surprisingly no influence of previous oviposition experience was found. In the tephritid species *Bactrocera tryoni* Prokopy *et al.* (1991) could show, that the females were able to learn the olfactory cues of the larval host fruit. Their first oviposition experience had a major impact on their further reproductive success. A similar increase of performance has been detected in several studies on different insect species (for example Dethier, 1982; Jaenike, 1990; Papaj & Lewis 1993; West & Cunningham, 2002). Experience had no influence on the ability of the flies to select for different host plants in both sexes of *U. cardui*. Freese & Zwölfer (1996) report that older females of *U. cardui* lay larger clutches, so this may be the only effect of experience in that species.

In the case of *U. cardui,* a specialised gallfly, the larval host plant is the activity centre for the larvae and the adult flies. It serves as a place to feed, mate and, of course, as an oviposition site. Thus nearly all behavioural patterns performed by the adult male and female *U. cardui* occur on the host plant. The important role of the host plant has also been stressed for other specialised fruit flies (for example Raghu *et al.*, 2002), indicating the unique role of the host plant for specialised herbivorous insects.

3.5 Acknowledgements

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4 The host plant recognition and the decision-time of *Urophora cardui* (Diptera: Tephritidae) in a stand of different plants

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The foraging process of a herbivorous insect is divided into several consecutive steps: 1) selection of a suitable host plant habitat, 2) selection of a suitable host plant and 3) selection of an oviposition site. The present work concentrates on the first and the second step in the foraging process of the specialised gall fly *Urophora cardui.*

Flies exhibited a very low response rate in the experiments presented here due to: 1) being tested singly, which decreased their motivation, 2) summer temperatures, which were unusually high for this region (>27°C), leading to a high mortality rate and higher disturbance of the flies during experiments, which were performed outside. Nevertheless, it was shown that individual variability in the responses of males and females to modified, artificial and natural host plants and to different arrangements of host plants, was higher than any possible sexual differences. Both sexes did not discriminate between different arrangements of thistles, but both sexes also avoided non-significantly several of the modified and artificial plants presented. This hints to a rigid host plant template.

The majority of flies made a decision for a particular host plant within the first five minutes of an experiment. The fact, that individual flies responded very fast in the experimental set-up indicates, that they were not disturbed by the artificial conditions in the flight cages, nor by the presence of an observer. No adjustment time to the experimental situation was necessary. This indicates that the results of this and all following chapters may mirror the foraging strategy under natural conditions quite well.

4.1 Introduction

The foraging process of herbivorous insects can usually be distinguished into different phases (Mangel & Roitberg, 1989; Prokopy & Roitberg, 1989; Roitberg *et al.*, 1982), which become more subtle from step to step (Zwölfer, 1968). In many herbivorous insects the host plant is also the rendezvous-place of the adult flies (Zwölfer, 1974). Therefore the search for a suitable host plant stand and a mating partner is linked (Mayhew, 1997; van Alphen & Vet, 1986; West & Cunningham, 2002; Whitham, 1978). The second step of the foraging process will be the choice of an individual host plant (Hassell & Southwood, 1978; Mangel & Roitberg, 1989; Prokopy & Roitberg, 1989). Once a suitable host plant has been selected the female still has to search for the most suitable host organ for oviposition and it has to adjust the clutch size to host plant quality (Charnov & Skinner, 1988). In each of these three steps the insect utilizes different cues of the habitat, the host plant and conspecifics (Beehler *et al.*, 1993; Fischer *et al.*, 2001). Usage of various cues also implies, that the insect relies on different sensory modalities, in order to perform an optimal choice (Prokopy & Owens, 1978;

Pyke, 1984). Optimal in this context indicates that the host plant quality should ensure the best possible larval performance in order to enhance the fitness of the herbivorous female (Nylin & Gotthard, 1998; Sitch *et al.*, 1988). The present chapter concentrates on the first step in the foraging process, the selection of a suitable host plant stand.

An individual's own fitness and through fitness of the next generation via larval performance depends on its behaviour while foraging (Craig *et al.*, 1989; Fritz *et al.*, 2000; Thompson, 1988; Via, 1986). Each of the three different steps during the foraging process determine the initial conditions of the following ones. Thus during each of these steps, optimal assessment of the environment is necessary for the female. If it fails in one task, the following ones will fail also. The selection of a suitable host plant patch has to be regarded as the first condition for foraging success. Hassell & Southwood (1978) define a patch (or stand) as the area in which an aggregation of food items (in the case of this study oviposition items) occurs and therefore an area which contains certain stimuli, that elicit a characteristic activity in the foraging insect, if the stimuli occur at a proper concentration. Rosenheim *et al.* (1989) define a host patch as the area over which variation in host density most strongly influences foraging. The most complex factor, which is important in host plant selection is the definition of the boundaries of a patch or a habitat (Feeny, 1990; Nordlund, 1994). Here the expression host plant stand is employed, which I define as the presence of several host plants and other vegetation in an area, which can clearly be assessed by the searching fly (a circle with a radius of approximately 2m with the female or male as the center).

In contrast to classical foraging theory, which was introduced for predators, which consume their prey (Charnov, 1976; Hassell & Southwood, 1978), the foraging of adult parasitoids involves the search for hosts they do not consume, but use for oviposition. Galling and mining herbivores also act like parasitoids (Rosenheim, 1996), they depend on high performance of their host during larval development. Various studies indicate that habitat quality is one of the important factors in determining if a species will be time or egg limited (Coley, 1983; Diaz-Fleischer & Aluja, 2003; Feeny, 1970). A high habitat quality is usually provided by the density of the host plants (Diaz-Fleischer & Aluja, 2003), since the more hosts are present the more likely a high number of hosts will be in a suitable condition for oviposition. For the quality not only the presence of host plants is important, but also the abiotic conditions of the habitat (Collinge & Louda, 1989): since characteristics of a certain habitat influence the performance of host plants, they influence indirectly the host plant selection of herbivores. Additionally an optimally foraging female should minimize its own mortality risk (Nonacs & Dill, 1991; Stoks & Johansson, 2000) and the mortality risk of its offspring (Mappes & Kaitala, 1995; Nomikou *et al.*, 2003). Thus a quite important factor, which influences host plant search of herbivorous insects or the host search of parasitoids is the occurrence of refuges (Jeffries & Lawton, 1984; Levin *et al.*, 1984). Induced host-plant structures like galls or mines in flower buds, fruits or on leaves especially often act as physical refuges (Ananthakrishnan, 1984). The presence of endophytes or bacteria is also important if they affect plant quality (Drew & Lloyd, 1987; Friedli, 2001; Kruess, 2002; Prokopy *et al.*, 1991).

Insect long-range detection of plants is likely to depend on visual cues and on olfaction simultaneously. The receptors designed to detect hosts from a certain distance are generally assumed to be mainly located on the antennae (Mitchell, 1988). Volatile perception will be influenced by windspeed. Van Alphen & Vet (1986) postulate that different insects use a variety of senses to locate their microhabitat, with olfaction being one of the most important ones. In members of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), chemical stimuli dominate the process of habitat and host location. They are initially attracted not by the host itself, but by a host-suitable habitat (Nordlund, 1994). A variety of abiotic factors influence this first step towards host location including humidity, light conditions, temperature, visual cues and probably also certain aural characteristics (Nordlund, 1994). These abiotic conditions are difficult to control in experiments: thus, it was not possible to gain information about the selection of habitat characteristics by *Urophora cardui.* The present chapter concentrates on the short distance orientation, for example on the detection of host plants (*Cirsium arvense*) from a distance of 1-2m.

Three different questions are posed:

1.) How rigid is the host plant template the individual flies use during the first phase of the foraging process? A comparison of different modified and artificial plants against their natural host plants, *C. arvense,* will reveal if they can also be attracted by models of their host plant.

2.) How long is the decision-time with regard to the host plant cues experienced?

3.) Does the artificial testing situation have an impact on the response of the tested flies?

Since the host plant is not only the oviposition site for the females of *U. cardui* but also the mating place for adults, the search of males for territories is included in the analysis.

4.2 Material and Methods

The biology of the study system is described in detail in chapter 2.1.2.

4.2.1 Origin of plants and flies, general rearing

Thistles and rhizomes were collected in early spring, when the first leaves of the plants were visible. Different habitats were used for plant collection: a wet meadow, a large border of a crop field and the bank of a brook. Several preliminary experiments showed that the females did not discriminate between plants of different origin, thus the results of plants from different origins were pooled in all experiments.

Thistle growing and synchronization with emergence of the flies was performed with a similar breeding strategy as used by Sakuth (1996).

Plants were potted in soil (5 parts neutral humic soil, 2 parts pumice, 2 parts lava, 1 part loess, 2 parts peat and 2500g Osmokote^{TM} (a long-time fertilizer)/1m^3 soil) and kept in the open to provide semi-natural conditions. In autumn and winter plants were grown in the greenhouse at L:D 15 (23°C):9 (18°C). The light conditions were achieved by an array of mercury lamps (Osram Power Star HQ I–TS 250 WINAL with a minimal light intensity of 400µEm^{-2}s^{-1}). The plants were watered twice a day to avoid drought stress, which would have affected female preference and larval performance. The thistles were repotted to larger pots after approximately two weeks growing and again two to four weeks later. The final pot size was three or five litres, according to plant size. Each plant was numbered, the height measured, and axillary buds and flower buds were counted before usage. The axillary buds were counted from the oldest leaves up to the top of the plant and numbered in the same direction. For multi-stemmed plants only the height of the largest shoot was recorded, but total shoot number was noted and all buds were counted as well. No thistle was used twice.

The *U. cardui* flies originated from several small populations within approximately twenty kilometres around Bayreuth, Germany. Some of the flies were part of the newly established laboratory population, although this experimental population could not be maintained without new flies from the field. The galls were collected in late summer and autumn and stored in the refrigerator until initiation of eclosure.

The flies emerged from these galls in Petri-dishes (ø 14 cm) and were then marked individually with a two colour-code (Hobbyline, Acryl - Colours in light green (10), blue (24), red (15), yellow (03) and orange (04)) on the wings. No interference of the marking with the behaviour was observed in preliminary comparisons. The same colour codes were used again, if new individuals emerged from galls after the original possessor had died.

Between the experiments the flies were held in plastic cages (volume: 6l, Fauna box by Savic, Belgium) lined with regularly moistened cellulose tissue. They were fed *ad libitum* with 50% honey in water solution or sucrose-solution 67.5% (w/v) (Freese & Zwölfer, 1996; McCaffrey *et al.*, 1994). Water was also provided *ad libitum*. A maximum of 15 flies was kept per box. Two of the boxes were for single sexes, containing only males or females, to have a stock of virgin, naïve individuals. In most cases prior to experiments the flies were not confronted with thistles, in order to enhance individual motivation.

4.2.2 Experiments analysing the host plant recognition and the decision-time of *U. cardui*

The experiments were conducted in large wooden field cages (2 x 1 x 1m). These were positioned outside the university building in an artificial thistle patch in order to enhance the motivation of the observed flies. All naturally growing plants inside the flight cage were removed (see the experimental design in chapter 3). The bottom of the cage was covered with artificial grass (Obi Germany). Other plants next to the field cages were mainly different species of common grass and other species of thistles. Thin white netting was used for the cage sides, so the flies could perceive the artificial thistle patch at least olfactorially. The observer was hidden by a black gauze curtain in order not to disturb the flies.

At one end of the cage a release point for flies was established with a 1m high stick and some artificial leaves as a platform at a height of approximately 0.70m (Fig. 4.1). According to experimental design, modified or artificial thistles or artificially arranged host plant stands were presented to the fly, 1.5m apart from the release platform. Table 4.1 shows a list of the different kinds of thistles which were used in the experiments. Each experiment lasted until the fly landed or otherwise after 45min. If possible, the same fly was confronted with different arrangements (but not twice with the same set-up to avoid pseudo-replication: Hurlbert, 1984; Wiley, 2003), but due to fly mortality, this was not always feasible. Each fly had at least four hours break between experiments, which the fly passed in the usual rearing plastic box (see chapter 2.2) together with conspecifics, food and water. Often experiments with the same fly were conducted on different days. Prior to the first experiment, the fly destined for testing was deprived of host plants for two days. During the experiments, no food was available in the flight cage, since this might have influenced the results.

Table 4.1: Modified and artificial thistles used in the experiments concerning host plant detection from a distance.

type	short name	description
natural	normal	a natural potted *C. arvense* of approximately 0.7m height
modified thistles	upside down	a potted *C. arvense* was hung from the roof of the cage, that the tip touched the ground; the pot held in place by two sticks
	bent down	the tip of a potted *C. arvense* was bend down with a green string, forming an U-Shape
	leaves down	the leaves of a potted *C. arvense* were tightly bound to the shoot with a green string, so the plant resembled a thickened shoot
dummies	paper	a paper-silhouette of a 0.7m high *C. arvense* plant was glued to a suitably sized stick and painted green with Acryl Buntlack seidenmatt, foliagegreen (Obi, Nr. IDD 170547 C RAL 6002)
	green plastic	green plastic leaves were fixed to a 0.7m high stick with green wire (Blumendraht). The stick was painted green with Acryl Buntlack seidenmatt, foliagegreen (Obi, Nr. IDD 170547 C RAL 6002)
	green-white	green-white plastic leaves were fixed to a 0.7m high stick with green wire. The stick was painted green with Acryl Buntlack seidenmatt, foliagegreen (Obi, Nr. IDD 170547 C RAL 6002), (Fig. 4.2)

Fig. 4.1: The point of release in the flight cage. The test-fly was released on the lower leaves.

Fig. 4.2: The artificial thistle green-white in a preliminary version with the stick not painted green and with short side shoots. A flight cage is in the background to the left.

Two different series of experiments were conducted: one designed to test the response to different modified or artificial thistles and one to test the response to different kinds of host plant stands. The latter experiments also included the presentation of artificial thistles. In the first series the test-fly was confronted with a single host plant (Table 4.1). If possible the response of the same fly to all different thistle varieties was tested. A walk or flight towards the thistle presented was counted as a positive response, if the fly remained on the release platform (Fig. 4.1) or moved along the walls, the roof or the bottom of the flight cage the behaviour was recorded as no response. These experiments were designed to estimate the rigidity of the host plant template used by *U. cardui.*

In the second experimental series, the flies were presented with four different arrangements of thistles. These experiments were designed to test the response of *U. cardui* to different sizes of host plant stands. In all of them 12 thistles were used, also including artificial thistles but none of the modified varieties (Table 4.1). In the first arrangement the plants were all put in a line, in a second set-up they were all clumped in a single patch in the middle of the flight cage, in the third, two patches with six thistles each and in the fourth, three patches with four thistles in each of the patches were arranged. These patches in the last two set-ups were at least half a meter apart, thus they were not all the same distance from the release point (between 1 and 2m).

These two experimental set-ups were designed to test host recognition by the flies and their search mode, if confronted with different plants in different stands. Additionally the time for individual *U. cardui* to respond was measured. Due to the small sample size in the experiments described above, time measurements of experiments, presented in the next chapter (chapter 5), were included too. The time individuals spend on a decision is related to the general search mode of *U. cardui* (part 4.1), thus I decided to include this pooled data in the present chapter.

Raw data were organized in an Access-database (Access 2000, Microsoft corporation). Preliminary table sorting and analyses were carried out in Access and in Excel (Excel 2000, Microsoft corporation); statistical analysis was performed in SPSS 10,0 (SPSS Inc.). If data were not normally distributed they were either transformed or an appropriate non-parametric test was used (Dytham, 1999; Sokal & Rohlf, 1995).

4.3 Results

4.3.1 General observations

Some of the modified thistles were found to be impractical for longer experimental series, leading to usage in only some experiments. These were the varieties upside down and bent down (Table 4.1). Upside down used to become unstable after one experiment or even earlier; bent down used to last only for approximately one hour, unless very strong string was used, but that appeared to disturb the flies. The paper variety (Table 4.1) was totally ignored by the flies and seemed to disturb them even if they chose a special patch, where normal thistles were also included. This variety was therefore only used in preliminary experiments and was excluded from the following analysis. I assume that the disturbance of the flies was due to movement of the leaves in the wind, since paper leaves move more and in a different way, than do natural thistle- or plastic leaves. Also not included in the following analyses was the variety leaves down, as the flies ignored it completely. To avoid statistics with almost only zeros, only the two plastic thistles were employed as a contrast to the normal host plant. Table 4.2 gives an overview of the frequencies with which the different types of thistles were used in experiments. In the experiments, which tested the response to different host plant stands, more than one thistle and more than one thistle type was used. In 10 preliminary experiments only artificial thistles were used. In 14 experiments concerning the arrangement of the thistles in a patch only natural host plants were utilized.

Table 4.2: Number of experiments in which the different kinds of thistles were utilized.

short name	number of experiments in which this type was included
normal	76
upside down	7
bent down	4
leaves down	10
paper	7
green plastic	31
green-white	31

Of 25 males tested in all the experiments, only three responded (12%). Eight females of a total of 47 females responded (17%). If the sexes were analysed together, the mean response rate is 15%. Fig. 4.3 shows the comparison between the number of individuals tested on 20 different days and the number of individuals that responded irrespective of the experimental design.

In nearly all cases, where flies flew to one of the presented thistles, movement around the chosen plant was performed by the fly directly after landing. In only two cases did the individual first rest and started moving from the landing point after several minutes.

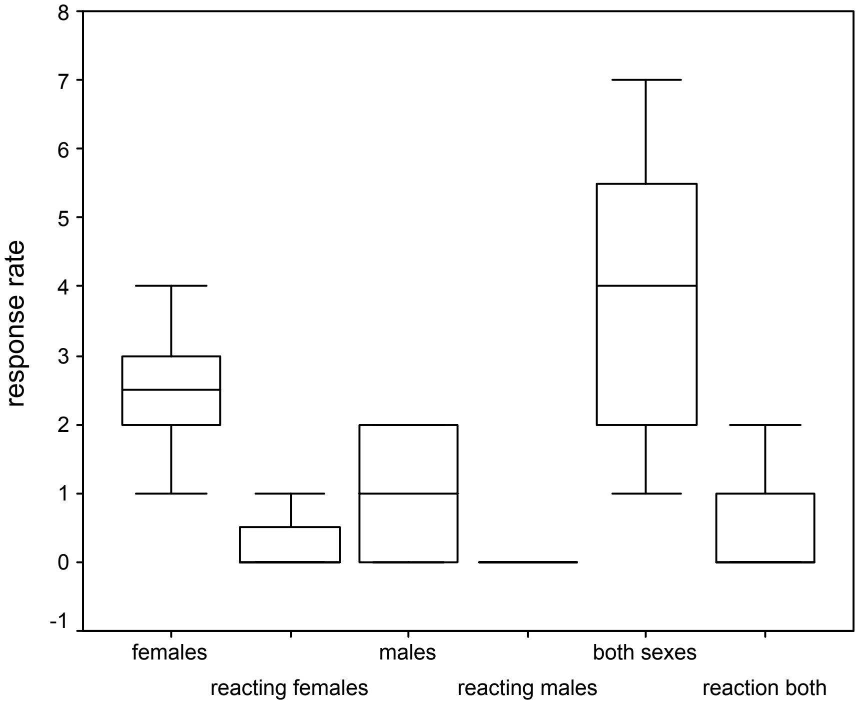

Fig. 4.3: Numbers of individuals tested and of individuals responding per day in different experiments performed in the flight cage over 20 days. Lines in the boxes=median; boxes=25-75%, whiskers=minimum and maximum response.

4.3.2 Selection of different types of thistles

Three different kinds of experiments were included here: in one only two artificial thistles with plastic leaves were presented (one green-plastic and one green-white, Table 4.1); in a second, only two normal thistles, and in the third one artificial (green-white) and one natural plant were offered. Only some flies responded at all, thus it is difficult to draw conclusions. Table 4.3 summarizes the percentage of response in the different set-ups. The connected design (one fly tested in different experimental set-ups) was not used in the analysis due to the low overall response. Kruskal-Wallis analysis revealed no difference between the number of animals responding to artificial thistles versus normal thistles, versus presentation of both types of thistles together ($H_{(2,15)\ males}$=0.952, p=0.621; $H_{(2,19)\ females}$=4.486, p=0.106). This applies if each sex was analysed alone and if sexes were pooled ($H_{(2,34)}$=3.277, p=0.194). There was no difference between the number of animals that responded in different treatments for the two sexes (Mann-Whitney-test: U=129,5, n=34, p=0.656), but due to the extremely low sample size it is possible that effects of the design are masked by the lack of motivation to react at all. If the experiments are compared to the results of the preliminary experiments (part 4.3.1), it is obvious that there was at least a trend to avoid artificial or modified thistles.

Table 4.3: Comparison of the response of males and females presented with different types of thistles (Table 4.1). The two females, which responded to presentation of a natural and an artificial host plant chose the natural host plant.

	♂			♀		
	n	$n_{responding}$	response [%]	n	$n_{responding}$	response [%]
two artificial host plants	5	1	20%	6	0	0%
two normal host plants	14	2	14%	25	6	24%
artificial and normal host plant	15	0	0%	7	2	29%

4.3.3 Selection for different patch sizes

No significant differences in the proportion of males and females responding in different set-ups (thistles presented in a line, or in one clutch, in two clutches, or in three clutches) were found ($H_{(3,15)\ males}$=1.741, p=0.628; $H_{(3,19)\ females}$=3.064, p=0.382). There was no difference between the two sexes in their response rate (Mann-Whitney-test: U=129.5, n=34, p=0.561). In these experiments the individual flies encountered not only different types of thistles but also different arrangements of these thistles. The response of males and females in the different set-ups is visualised in Fig. 4.4. The high standard deviation indicates high individual variability in these experiments (see chapter 3). In general, the overall response rate was low, as in the first series of experiments. Thus it is possible that there were effects of the arrangement on the response, but these were masked by overall poor motivation. In Fig. 4.4 a similar trend in the response to different presentation of thistles was shown in both sexes. The variability in response was highest in the experimental set-up where the host plants were presented in one cluster. Response to one of the plants seemed to be lower in both sexes, if the thistles were presented in one line.

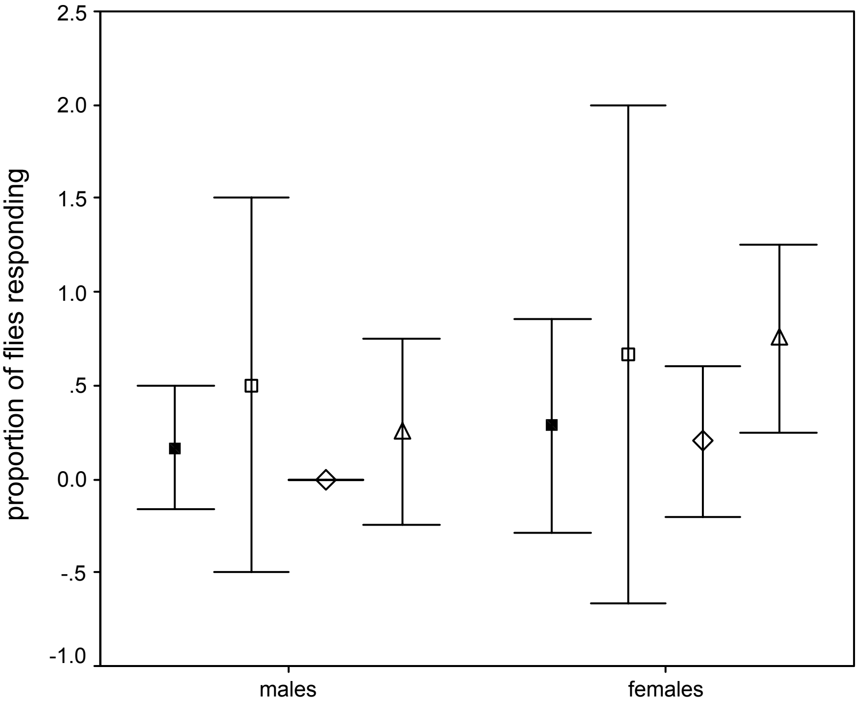

Fig. 4.4: Response of males and females towards different arrangements of thistles: ■ in a line ($n_{males}=6$, $n_{females}=7$), □ one cluster ($n_{males}=2$, $n_{females}=3$), ◊ two clusters ($n_{males}=3$, $n_{females}=5$), Δ three clusters ($n_{males}=4$, $n_{females}=4$). Y-axis: proportion of flies responding (the number of responding animals was divided by the number of tested animals) and the SD.

4.3.4 The time needed to decide on a certain host plant

Experiments in which the time until the first response of male or female *U. cardui* flies was measured, were pooled. If the resulting distribution of decision-time is plotted in a graph (Fig. 4.5), it is quite obvious, that the data were not normally distributed but skewed to the left (K-S test: Z=2.101, n=66, p<0.001), thus the decision was performed within the first minutes.

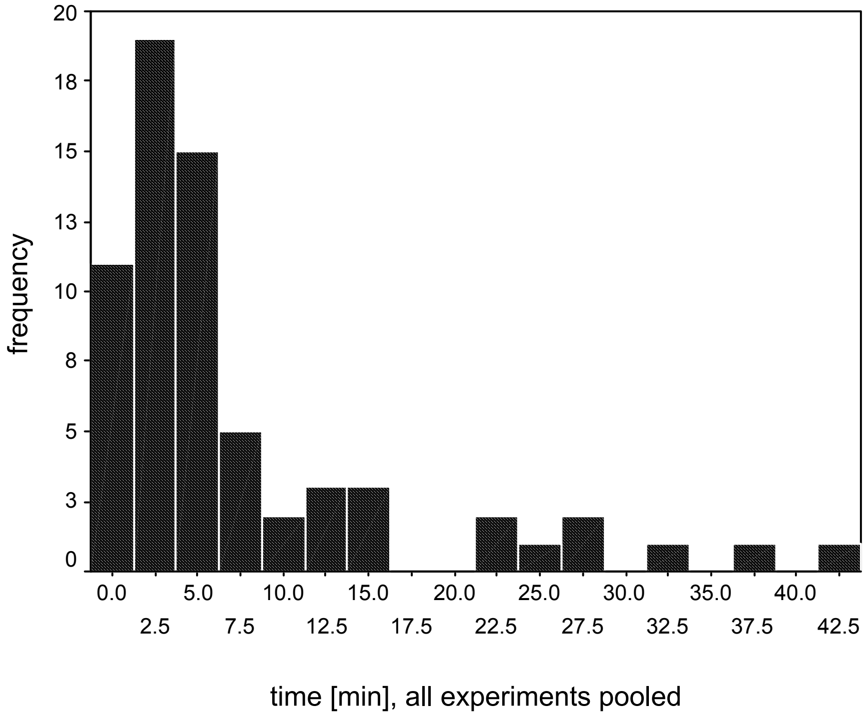

Fig. 4.5: Histogram of the time needed by the flies to decide until they choose a host plant. Data of different experimental set-ups were pooled.

There was no difference between males and females in their response time (Mann-Whitney-test: U=486.5, n=66 (28 males, 38 females), df=1, p=0.56) (Fig. 4.6). In both sexes decision-time to land on a thistle had a distinct peak at 2.5 minutes; a few more landing decisions (39.4%) occurred after the first five minutes. If the times until a decision is made were grouped into five-minute classes, 40 individuals decided within the first five minutes, ten in the following five minutes and 16 individuals needed more time until they chose a suitable host plant. Of all the 66 animals, which were included in this analysis, 57 chose one of the plants within the first quarter of an hour. So as time passed, the probability that the individuals decided to choose a plant declined markedly. This was at least true in an artificial experimental set-up. It shows, that for the great majority the of the flies, motivation was not enhanced by the time an experiment lasted. Either the flies were motivated and chose nearly immediately or they were not and they remained in that condition. These data also show that the motivated flies did not seem to need time to adjust to the experimental set-up.

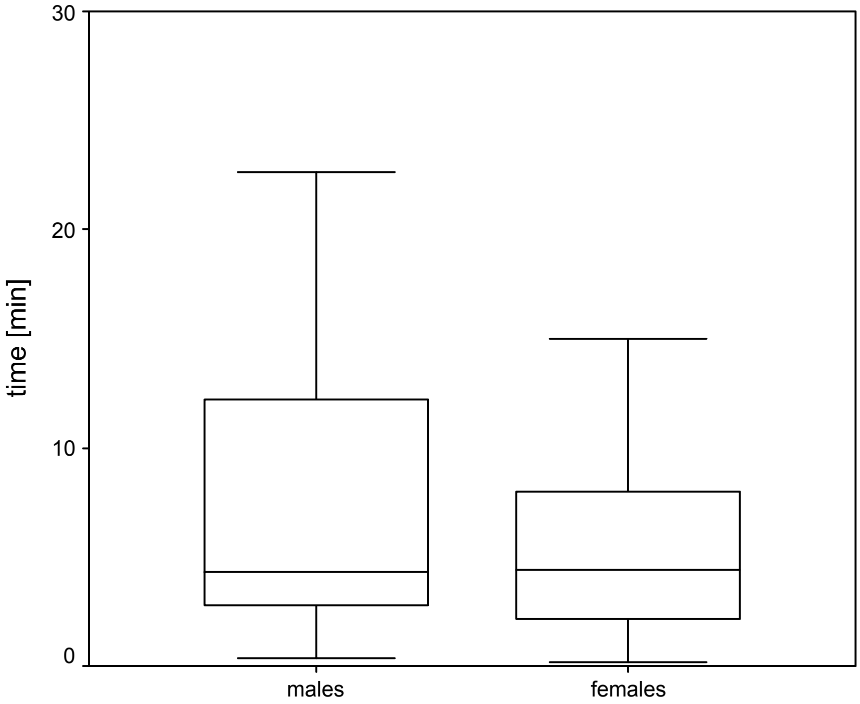

Fig. 4.6: Comparison of the time required until males n=28) and females (n=38) of *U. cardui* decided on a particular host plant. Data were pooled from different experiments. Lines in the boxes=median, boxes=25-75%, whiskers=minimum and maximum time until a decision was performed.

There was a significant influence of the presence (or absence) of artificial and/or changed plants on the time the flies spent until they decided on one of the plants (Kruskal-Wallis analysis: $H_{(2,14)}$ =7.5, p=0.023). In tests with dummies and/or modified plants, the flies reached a decision faster than in the treatments in which only normal host plants were presented. The reason for this behaviour may have been that the number of acceptable thistles declined. If other plants were also present, the fly needed less time to compare the host plants present, i.e. the artificial host plants (which may be comparable to non-host plants in natural environments) can be rejected faster. Fig. 4.7 visualizes this relationship between decision-time and treatment set-up. Only one individual (a male) responded in an experiment in which only dummies (artificial) were presented. Thus the decision-time measured in this single response cannot be compared to the other experiments, where more individuals responded. In general, in these experiments, individual variability was also high. There was no influence of the different patch sizes on the decision-time (Kruskal–Wallis analysis: $H_{(3,11)}$=1.8, p=0.629).

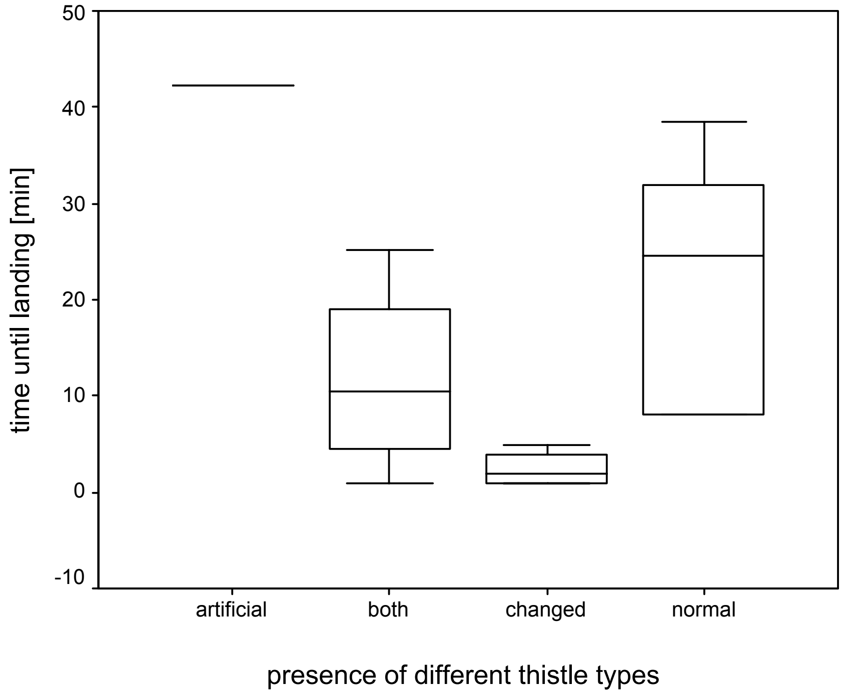

Fig. 4.7: Time until a decision for one of the presented thistles was made in relation to the presence of artificial thistles (artificial, note that n=1individual), changed host plants (changed, n=4 individuals), artificial, natural and changed plants (both, n=4 individuals) and only normal host plants (normal, n=6 individuals). Lines in the boxes=median, boxes=25-75%, whiskers=minimum and maximum response times.

4.4 Discussion

The most striking result was the low response rate independent of the experimental design. As found later, individuals of both sexes of *U. cardui* were more strongly motivated if tested together with several males and females of the same species. As Hölldobler (1999) points out, motivation has a major impact on the behaviour of individuals especially in situations where response to specific cues is necessary. It is not yet clear if the enhanced motivation in the presence of conspecifics was an effect of grouping (lower predation and parasitation risk (Shiojiri & Takabayashi, 2003)), or if the flies were influenced by the motivation of conspecifics, or simply by the presence of the other sex. Nevertheless, the low response rate implies that the results of the present chapter should be regarded with caution. A high mortality rate due to very high summer temperatures during the experimental period led to a low sample size in several experiments. Additionally the high temperatures may have also led to lower motivation of the individuals. Thus, these three effects may obscure genuine differences.

Nevertheless general observations on various artificial thistles (upside down, bent and leaves down, Table 4.1) and dummies (paper, Table 4.1) indicated that *U. cardui* tend to avoid modified and artificial host plants. This indicates again the rigid host plant template this specialised gall fly uses during selection of larval host plants (see chapter 3.3.2). If modified and/or artificial thistles were presented together with normal hosts, *U. cardui* selected the normal hosts. The arrangement of these thistles in different patch sizes had no influence on the response. Males and females behaved similarly in the experimental set-ups. There was a high individual variability (see chapter 3), which may have masked sexual differences and the effects of the different patch sizes.

Generally the decision takes place in the first five minutes. The flies did not seem to need time to adjust to the experimental set-up. After the first fifteen minutes a response became unlikely. Thus, in all further experiments, the duration of the test was shortened to half an hour. The motivation of the flies did not depend on the experimental set-up: intrinsic factors are most likely to cause individual variability in the motivation to select a certain host plant (Cunningham & West, 2001).

In mixed set-ups with a combination of modified, artificial and normal host plants the flies decided faster on one of the hosts. It can be assumed, that they ignored the modified and artificial plants after a short examination. Thus, they had a smaller number of plants available as potential hosts, which shortened the comparison time. In insects where a host shift may be costly, as is the case in specialised galling species only small possibilities for less discrimination exist (Larsson & Ekbom, 1995). In *U. cardui* only lowering of the acceptance-threshold to closely related host plant species (*C. creticum*) could be observed (H. Zwölfer unpublished data). The requirement of more time for a decision if only natural hosts are presented leads to the assumption that females perform faster, if not too many suitable host plants are present in the respective host plant stand. If the host plants dominate the stand the insect has many possible hosts available, thus needing more time to select the most suitable one. But if the host plants are present only in small numbers and/or are inconspicuous (due to a high grass vegetation for example), the insect may have difficulties in finding and recognising the host plants against the background. If hosts and non-hosts occur in mixed stands there are fewer plants that the fly has to assess the quality of. Roitberg *et al.* (1982) showed, that in the case of the tephritid fly *Rhagoletis pomonella* the females were more selective in their choice of host fruit for oviposition as the density of host fruit increases.

Host plant quantity and the presence of host plant cues affect the foraging behaviour of the flies. For example *R. pomonella* move faster in host plant patches permeated with host odour; additionally their movements were more direct (Aluja & Prokopy, 1992). If flies were exposed only to a point source of odour, they flew to the source if they perceived the odour. Prior to this response they exhibited random searching flights, like flies exposed to clean air. Habitat characteristics like vegetation density have an important influence on wind trajectories and therefore on the direction of host plant volatiles. It is assumed, that long-range searching patterns are not only influenced by direct volatiles of the host, but are also a function of other olfactory cues of the habitat (Robert, 1986 and references within). In the case of *U. cardui* this was only tested in preliminary experiments, which suggested that presence of regularly watered host plants around the observation cages enhanced motivation of the flies. Also as shown here host plant density affected the selection time and may thus affect the foraging success of individual *U. cardui*.

There are hardly any studies concerning the ability of herbivorous insect to judge the abiotic conditions of the habitat of the host plants. *U. cardui* is restricted to a small variety of habitats, which makes it likely that the females are able to assess at least the water and shading conditions of the habitat (see chapter 2.1.2). During gall initiation the larvae also depend on a high amount of nutrients: nutrient levels influence not only the herbivore but also the other trophic levels (Mayntz & Toft, 2001). Vice versa, the presence of herbivory or a plant gall will influence the host plant. The impact on the plant will vary according to local conditions: competition with neighbouring plants or nutrient availability will affect the ability of the plant to compensate for the effects of galling (Maschinski & Whitham, 1989). If the plant is negatively affected by such effects, this will again in turn influence the herbivorous insect (Clancy & Price, 1986). The better the individual fly is able in advance to judge plant performance the higher its own reproductive fitness will be.

The fact, that *U. cardui* males and females did not react in experiments, where they were only able to select artificial or modified host plant hints that they perform a decision while entering the habitat. They spent less time in moving from plant to plant in the host plant stand and in sampling the plants they encountered, they already performed a preselection and did not visit other plant species, which are unsuitable for larval development. This result is very similar to the analysis of selection time in two sister taxa of *U. cardui, U. quadrifasciata* and *U. jaceana* (Burkhardt & Zwölfer, 2002). Females of the generalist species *U. quadrifasciata* needed on average three times longer than females of the specialist *U. jaceana* to select a suitable plant and oviposit on it, although the latter lays egg clutches while *U. quadrifasciata* deposits single eggs. This indicates that specialised insects have a better ability to judge the suitability of host plants quite fast, compared to generalist species (Askew, 1980; Bernays, 1999; Bernays & Chapman, 1994; Janz & Nylin, 1997; Prokopy & Owens, 1978). The time costs are thus lower in specialised species and ensure higher reproductive rate per unit search time. However, usually these specialised species are subject to a higher demand for synchronization with their host plant (Brewer & Skuhravý, 1980; Burkhardt & Zwölfer, 2002), which decreases the reproductive rate over the life-time of the insect.

4.5 Acknowledgements

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5 How do males and females of the specialized gall fly *Urophora cardui* (Diptera: Tephritidae) select for a particular suitable host plant?

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Generally it is assumed that herbivorous insects use different senses in recognition and selection of host plants. Several different cues of the host plant together provide a reliable information on host plant quality. The usage of visual and olfactorial cues were analysed in the specialised gall fly *Urophora cardui* searching for its host plant *Cirsium arvense.*

The plant structure and its developmental stage had an impact on the host plant selection of *U. cardui*. Whereas the height of the plant was of minor importance, the amount of axillary and flower buds had a significant impact. Plants with a moderate number of side shoots, indicating the presence of many suitable axillary buds, were preferred. Plants with more than 15 flower buds were avoided.

Experiments in the wind tunnel showed the usage of different senses during host plant selection. Males of *U. cardui* could use visual or olfactory information to select for a territory. Plants marked by conspecific males were preferred, indicating, that the costs of a fight with the resident male are taken into account rather than facing the risk of predation by spiders.

In contrast, females did not respond to the odour of the host plant, they used only visual information for recognition of their host plant. Plants, which had been previously marked by a male were preferred. The marking substance of the males signals not only presence of a mating partner, but also enemy-free space, where the female will be able to deposit eggs undisturbed by spiders. Discrimination between marked and unmarked host plants was performed on olfactory cues alone. Some preliminary electrophysiological experiments corroborated these results. They highlight the importance of the preselection performed by the males of *U. cardui*.

5.1 Introduction

The host plant selection of herbivorous insects is a complex task, which generally involves the usage of different sensory modalities to obtain more reliable information of the host plant and its suitability (Bernays & Chapman, 1994; van Alphen & Vet, 1986; West & Cunningham, 2002). The more different cues can be percepted and processed, the better the insect will be able to judge host plant quality (Dethier, 1982; Priesner, 1986). The process of host selection by herbivorous insects can be divided into various steps: 1.) habitat selection, 2.) selection of a particular plant, 3.) selection of a suitable host plant organ (see chapter 4.1; Roitberg, 1985). The recognition and selection of host plants do not only involve the usage of different sensory modalities in all these tasks, but also the integration of neural and metabolic events (Bernays, 1999). These are for example sensing and encoding of the informational input (Carlsson & Hansson, 2003), decoding mechanisms in the central nervous system (Hansson, 2002), and the intrinsic state of the searching insect, for example its nutritional balance (Louw & Nicolson, 1983), experience (Cunningham & West, 2001), mating state (Robacker *et al.*, 1985), age (Begon & Parker, 1986) or previous oviposition success (Dethier, 1982).

Host plant recognition and selection by herbivorous insects is usually performed with the usage of host plant templates (see chapter **4.1**, chapter **4.4**; Krebs & Davies, 1993). This signifies, that a particular stimulus in the external world (or a combination of different stimuli) matches a model in the neural world and that upon the occurrence of congruency the relevant behavioural sequences are performed (Dethier, 1982). These host templates are also named cognitive maps after Tinbergen (1934). The investigation of the various host plant signals is an important area of science (see for example Aluja & Prokopy, 1993; Beehler *et al.*, 1993; Dethier, 1982; Fischer *et al.*, 2001), although they have been studied since the 1930s. It is assumed that the host plant quality is indicated by different cues (Myers, 1985). The host plant quality has a major impact on larval performance (Baylis & Pierce, 1991) and thus on its reproductive success (Fletcher & Prokopy, 1991). Fertilization of plants may for example change the leaf colour, which can be discriminated by herbivorous insects, as was for example shown in the case of the butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae) (Baylis & Pierce, 1991).

The above-mentioned usage of different sensory modalities during the complex host plant selection and recognition implies that not only receptors on the antennae play a role, but also receptors on the mouth parts, the tarsae or the ovipositor (Robert, 1986). The information perceived consists of volatile profiles (Aluja & Prokopy, 1992), visual information (Scholz *et al.*, 1997), and contact kairomones (Dutton *et al.*, 2000) together, which interact in their influence on the searching insect (Beehler *et al.*, 1993). Nevertheless, most studies focused only on one aspect of this selection process and, furthermore, even on one part of the different involved sensory inputs, therefore the selection process as a whole is not well understood so far (Robert, 1986). Dipterans are well known for their highly developed visual system (Agee, 1985; Agee *et al.*, 1982), thus many studies concentrate on the investigation of visual cues (Green *et al.*, 1994; Moericke *et al.*, 1975; Prokopy, 1968; Roitberg, 1985; Sharma & Franzmann, 2002). For *Ceratitis australis* (Diptera: Tephritidae) it could be shown that physical characteristics of the host plant like shape, size and colour attract females from the distance (Pittara & Katsoyannos, 1992), while the surface structure of the plant elicited probing behaviour (Pittara & Katsoyannos, 1992). In tephritid pest species, for which traps had to be constructed, there are also some olfactorial studies (Baker *et al.*, 1990; Fein *et al.*, 1982; Frey *et al.*, 1990; Ngi-Song *et al.*, 1996) for example on *Rhagoletis pomonella*, the apple maggot fly (Carle *et al.*, 1987). During its main activity period (July-September), there occurs a high similarity of the olfactorial profile of various apple cultivars and hawthorn (*Crataegus coccinea*) the native host of *Rhagoletis pomonella* (Carle *et al.*, 1987). This led to an expansion of the host range of the apple maggot fly (Carle *et al.*, 1987). In the present study of *U. cardui* the role of visual and olfactorial stimuli simultaneously of the host plant for a specialised gall fly were investigated.

Burkhardt & Zwölfer (2002) were able to show, in their intensive study on the influence of host plant quality on the selection of two different *Urophora* species, that the species with the less complex galls was less selective. Additionally this species was able to use the plant during a broader temporal window. *U. jaceana* however, which forms a complex gall with a vascular system, was able to discriminate between plants with a different nutritional supply. The galls induced by *Urophora cardui* on the creeping thistle, *Cirsium arvense*, are even more complex than the ones induced by *U. jaceana* (see chapter **2.1.2**), thus it can be assumed that flies of this species should be even more selective with regard to suitable host plants and especially their quality.

Other experiments (see chapter 3) on the behaviour of males and females of *U. cardui* indicate that the territories of the males might influence the selection for host plants suitable for oviposition by females. Male *U. cardui* establish territories on the larval host plant, which they mark with a secretion of their rectal gland (Frenzel *et al.*, 1990). This marking of the plant may signal the presence of a mating partner to the females and possibly also a spider-free plant (see chapter 2.1.2). If a spider is on the plant they select as a territory, generally the spider catches them within seconds (H. Zwölfer pers. comm., personal observations). A marked host plant thus indicates, that the male had time enough to perform marking of its territory (see chapter 2.1.2 and chapter 3.3.1.2).

In the first series of experiments, presented in this chapter, the preference of *U. cardui* females for several characteristics of their host plant was investigated. Height, branching level, which indicates also the number of axillary buds, and the number of flower buds, altogether indicate the developmental state of the plant.

In a second experimental series, performed in a wind tunnel, the influence of different senses during the complex selection behaviour of *U. cardui* was tested. According to the results in the first experimental series it was ensured that the plants presented in the wind tunnel were suitable for oviposition. Additionally the impact of the marking substance (Frenzel *et al.*, 1990) was investigated.

5.2 Material and Methods

The biology of the study system is described in detail in chapter 2.1.2.

5.2.1 Origin of plants and flies, general rearing

Thistles and rhizomes were collected in early spring, when the first leaves of the plants were visible. Different habitats were used for plant collection: a wet meadow, a large border of a crop field and the bank of a brook. Several preliminary experiments showed that the females did not discriminate between plants of different origin, thus the results of plants from different origins were pooled in all experiments.

Thistle growing and synchronization with emergence of the flies was performed with a similar breeding strategy as used by Sakuth (1996).

Plants were potted in soil (5 parts neutral humic soil, 2 parts pumice, 2 parts lava, 1 part loess, 2 parts peat and 2500g Osmokote^{TM} (a long-time fertilizer)/1m^3 soil) and kept in the open to provide semi-natural conditions. In autumn and winter plants were grown in the greenhouse at L:D 15 (23°C):9 (18°C). The light conditions were achieved by an array of mercury lamps (Osram Power Star HQ I–TS 250 WINAL with a minimal light intensity of 400µEm^{-2}s^{-1}). The plants were watered twice a day to avoid drought stress, which would have affected female preference and larval performance. The thistles were repotted to larger pots after approximately two weeks growing and again two to four weeks later. The final pot size was three or five litres, according to plant size. Each plant was numbered, the height measured, and axillary buds and flower buds were counted before usage. The axillary buds were counted from the oldest leaves up to the top of the plant and numbered in the same direction. For multi-stemmed plants only the height of the largest shoot was recorded, but total shoot number was noted and all buds were counted as well. No thistle was used twice.

The *U. cardui* flies originated from several small populations within approximately twenty kilometres around Bayreuth, Germany. Some of the flies were part of the newly established laboratory population, although this experimental population could not be maintained without new flies from the field. The galls were collected in late summer and autumn and stored in the refrigerator until initiation of eclosure.

The flies emerged from these galls in Petri-dishes (ø 14 cm) and were then marked individually with a two colour-code (Hobbyline, Acryl - Colours in light green (10), blue (24), red (15), yellow (03) and orange (04)) on the wings. No interference of the marking with the behaviour was observed in preliminary comparisons. The same colour codes were used again, if new individuals emerged from galls after the original possessor had died.

Between the experiments the flies were held in plastic cages (volume: 6l, Fauna box by Savic, Belgium) lined with regularly moistened cellulose tissue. They were fed *ad libitum* with 50% honey in water solution or sucrose-solution 67.5% (w/v) (Freese & Zwölfer, 1996; McCaffrey *et al.*, 1994). Water was also provided *ad libitum*. A maximum of 15 flies was kept per box. Two of the boxes were for single sexes, containing only males or females, to have a stock of virgin, naïve individuals. In most cases prior to experiments the flies were not confronted with thistles, in order to enhance individual motivation.

5.2.2 Morphological characters of thistles selected for oviposition

218 thistles, which were used to maintain a laboratory breed of *U. cardui,* were measured in height; the axillary buds and the flower buds were counted. The axillary buds were counted and numbered from the oldest leaves up to the youngest ones at the top. If the plant had more than one shoot, only the height of the largest shoot was measured, but total shoot number was noted and all axillary and flower buds were counted. Each thistle was used only once. The height, the number of axillary buds (in the following also labelled branching) as well as the number of flower buds were assigned to three classes each, as shown in Table 5.1.

The flies had always access to ten to twelve different thistles in a flight cage (2 x 1 x 1m), for at least two days during their life. In the analyse of the different characteristics of the thistles all galls were used including 31 galls, of which no adults emerged since a gall only develops, if at least one L2 larva was present (Lalonde & Shorthouse, 1985). The occurrence of galls in relation to the morphological attributes of the thistle described above were analysed via chi-square tests in SPSS 10.0 (SPSS Inc.). The expected values for the chi-square test were calculated according to the distribution of the amount of thistles in the different classes of the sub-population analysed here (Table 5.3).

Table 5.1: Definitions of the classes used for the description of the attributes height, branching and flower bud amount of the host plants, which together provide information about the developmental stage of the plant.

	height [cm]			branching			flower bud number		
from-till	0-25	26-50	>50	0-10	11-20	>20	0-15	16-30	>30
class	small	middle	large	weak	moderate	strong	few	some	many

5.2.3 Experiments in the wind tunnel

Several different experiments were conducted in a wind tunnel, to allow 1.) constant environmental conditions during experiments, 2.) a better perception of olfactory signals by the flies and 3.) experiments during winter.

The wind tunnel (Fig. 5.1, Fig. 5.2) was similar to that used by Rojas (1999), it measured 1.6 x 0.75 x 0.75m meters. A Fischbach speed controller fan (D340/E1, FDR32, Neunkirchen, Germany) pushed air through the tunnel. The incoming air was cleaned by four charcoal filters (145 x 457mm, carbon thickness 16mm, Camfil Farr). In preliminary experiments a wind speed of 0.55m/s was determined as suitable. This wind speed did not disturb the flies, but was not too low on the other hand (see Aluja & Prokopy, 1992; Zhang *et al.*, 1999). The wind tunnel was originally designed for moth experiments. Preliminary experiments showed that it was too large for *U. cardui*, therefore only the first half of the flight chamber (0.8m) was used for experiments, the other half was left vacant.

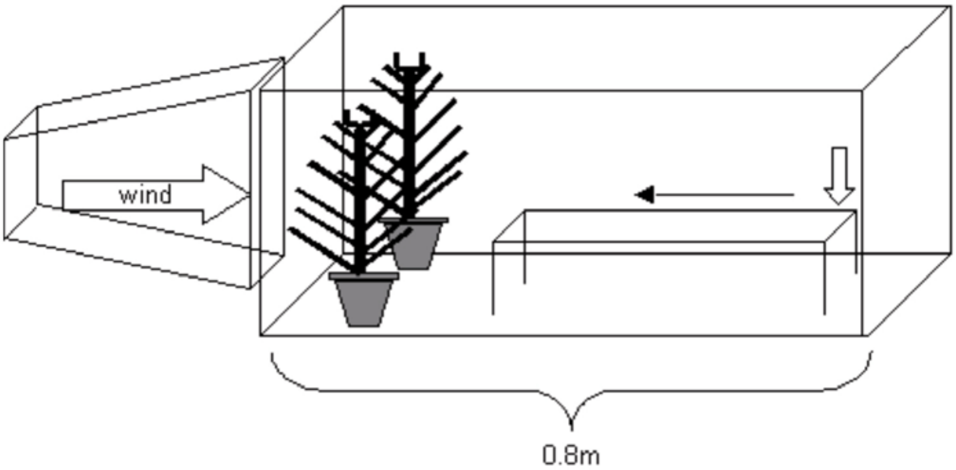

Fig. 5.1: Schematic drawing of the wind tunnel. The motor on the left pushed a constant air stream into the flight chamber on the right. The motor is separated from the flight chamber by a fence. At the left end of the wind tunnel the air stream can leave the system through a net. Inside the wind tunnel on the right is the platform (see below), the white arrow signifies the point of release, the black arrow signifies the direction of the expected movement.

The wind tunnel stood in a separate room, where no disturbance occurred. The windows of that room were oriented to the south and the east, thus the flies had generally natural sunlight during experiments. This enhanced their motivation as determined in preliminary experiments. The temperatures in the room varied between 23°C and 27°C. The experiments were conducted between 09:00 and 16:00 (CEST), as during these hours the flies seemed to be most active. On very sunny days the windows adjacent to the wind tunnel were covered with rollups.

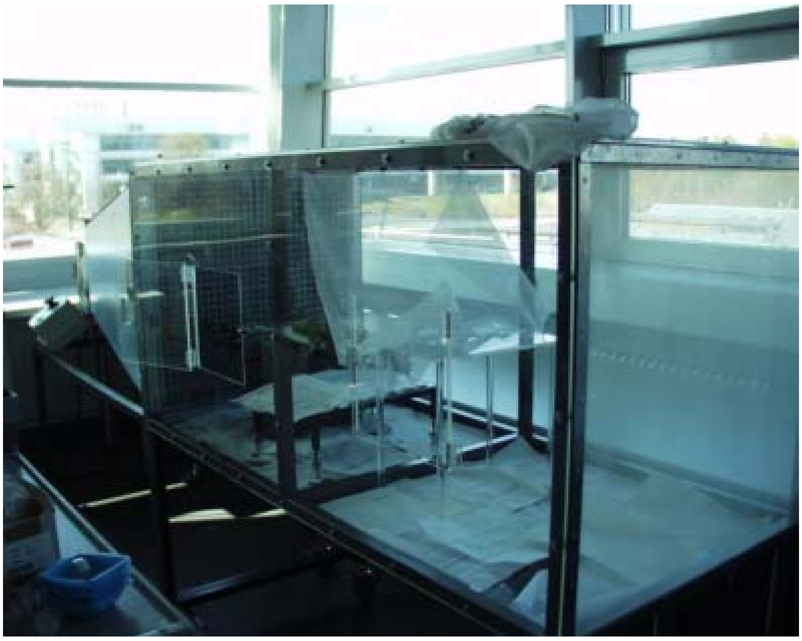

Fig. 5.2: View of the wind tunnel, in which experiments analysing the usage of different senses were conducted. The motor with the charcoal filters is on the left, on the right the flight chamber. In the rear the large windows are visible.

Preliminary results showed that the flies are in general more motivated if conspecific males and females were present. Thus between four and ten individuals were tested together. Usually both sexes were tested simultaneously. One experiment lasted 30 min (see chapter 4.3.4). Between tests in different set-ups the flies were transferred into their storing boxes (see chapter 2.2). To ensure, that the flies remained motivated to select a host plant, generally the different tests with the same fly were conducted on different days. In the wind tunnel no food or water was provided, to avoid influence on the outcome of the experiments by presenting additional olfactory and visual stimuli. The flies were released on a plexiglas table measuring 30 x 15cm, since they seemed to prefer walking to flying, as preliminary experiments indicated (see also Zhang *et al.*, 1999). The transparency of the table disturbed the flies in preliminary experiments. To eliminate this disturbance the table and the bottom of the cage were covered with paper (C. Linn pers. comm.). The table will be named simply platform henceforth. The flies were observed with a binocular (Fernrohrlupe, mobilux far, Eschenbach, Nürnberg Germany) during the whole experiment, since a greater distance on the part of the observer to the wind tunnel could be maintained and the flies were thus not disturbed by the experimenter’s presence. The continuous movement to the left wall, where the cues of the host plant were presented (Fig. 5.1), was defined as a positive response. In all experiments two thistles were used, since that enhanced the motivation of the flies (see chapter 5.2.3). For every responding individual the time until a positive response was stopped and recorded (see chapter 4.3.4).

5.2.3.1 Discrimination between a host and a non-host plant

In one set-up a thistle and another plant species (*Sonchus asper* (Asteraceae)), which was common in the natural habitats where plant galls were collected, were presented together. Here the flies could use visual and olfactory cues simultaneously. 62 males and 83 females were tested comparing a host to a non-host plant. The preference for one of the plants was analysed via Wilcoxon Tests (Dytham, 1999) in SPSS (10.0 SPSS Inc.).

5.2.3.2 Visual and olfactory discrimination of the host plant

Four of the experimental set-ups in the wind tunnel were designed as connected experiments (one fly was tested in all set-ups, Table 5.2): in one test only visual cues were presented to the flies (Fig. 5.3, the thistles were presented behind a plexiglas screen), in another one only olfactory ones (Fig. 5.4, Fig. 5.5 the thistles were presented behind a gauze curtain), the third was a positive control, where visual *and* olfactorial stimuli were presented (Fig. 5.1) and the fourth a negative control of the olfactory set-up (only the gauze curtain was presented to the flies). The details of the different treatments in this series are summarized in Table 5.2. Animals reacting to the gauze curtain (Table 5.2) alone were not counted as reacting in the olfactorial experiment. Especially the females seemed to be attracted by the gauze curtain, therefore additional olfactorial experiments were performed with another experimental design (see chapter 5.2.3.3).

Each fly had to participate in each of the different experimental set-ups, to make comparisons between the individuals possible and exclude individual variability (see chapter 3). No individual was used in a single set-up twice to avoid pseudoreplication (Cottenie & Meester, 2003; Hurlbert, 1984; Oksanen, 2004). The sequence in which the flies passed the different experiments was random. Since experiments were usually conducted on different days, in order to give the flies a break, not all flies survived until they had passed all different set-ups. Only the negative control was always tested at the same day as the presentation of olfactory cues. In the statistical analysis only data of those flies, which passed all set-ups were included (Dytham, 1999; Sokal & Rohlf, 1995). Table 5.2 gives an overview of the numbers of males and females tested in each set-up. Statistical analysis was performed in SPSS 10.0 (SPSS Inc.). Friedman ANOVAs were employed to check for globally significant effects and the Wilcoxon-Wilcox post-hoc test for the localisation of significant effects was calculated after Sachs (1999).

Table 5.2: Experimental set-up of the four different connected experiments.

treatment	positive control (both)	visual detection	olfactorial detection	negative control
number of thistles	2	2	2	0
Set-up	Thistles were presented on the left side of the platform.	Thistles were presented behind a plexiglas screen on the left side of the platform.	Thistles were presented behind a curtain of gauze on the left side of the platform.	Only a gauze curtain was presented on the left side of the platform.
tested males	86	44	53	53
tested females	102	117	112	112

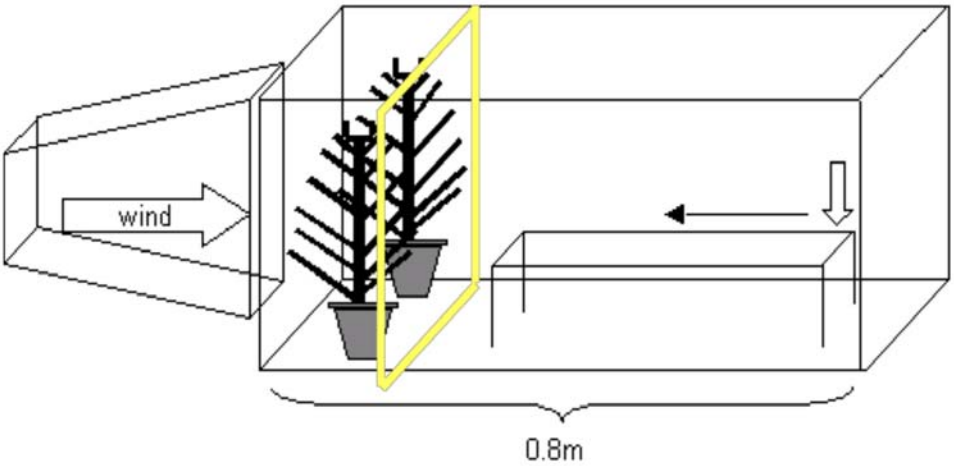

Fig. 5.3: Presentation of visual cues in the wind tunnel. The motor is on the left, the flight chamber on the right. Two thistles were presented in the air stream. Yellow lines=plexiglas screen, table=platform, white arrow=releasing point, black arrow=expected movement. No olfactorial stimuli could pass through the screen. Due to the low wind speed it can be excluded, that swirls occurred at the edges of the screen.

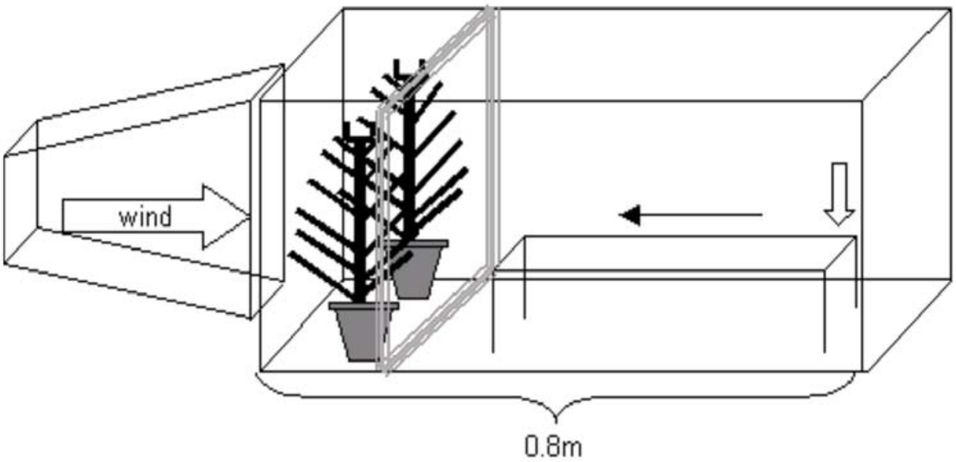

Fig. 5.4: Presentation of olfactory cues in the wind tunnel. The motor is on the left, the flight chamber on the right. Two thistles were presented in the air stream. Grey rectangle=gauze curtain, table=platform, white arrow=releasing point, black arrow=expected movement. As determined in preliminary experiments no visual stimuli could be percepted through the gauze net.

Fig. 5.5: Presentation of olfactory cues in the wind tunnel. On the left one of the two presented thistles is visible behind the gauze curtain. On the right is the platform.

5.2.3.3 Additional olfactory experiments

In order to corroborate the results of the connected experiments concerning the olfactory detection of a host plant by males and females of *U. cardui* another series of experiments with a more sophisticated design was conducted in the wind tunnel. These were also combined experiments, meaning that each male and female had to participate in an experimental run and in a negative control. This time the plants were not presented behind a gauze curtain, but in the chamber directly after the wind generator. This chamber was separated from the flight chamber by a fence. It was not possible to present potted plants, since the wind tunnel access opening was too small for the pots (see chapter 5.2.1). The thistles were carefully removed out of their pot and positioned in a glass with water. One thistle was used for one experimental run only, otherwise they tended to wild. In the negative control only the glass with some soil and water was presented.

As in the other wind tunnel experiments the flies were tested for 30 min on the platform. Between six and ten individuals were tested simultaneously. Continuous movement up to the chamber wall was counted as a positive response. Often the responding flies tended to climb the wall until the height of the thistle in the glass. I assume that they were searching for the thistle they were able to percept olfactorially. Due to a high mortality in the laboratory population at the same time it was only possible to test 34 males and 32 females in this way. Differences in the response in the experiment and the negative control were analysed using Wilcoxon tests, occurrence of a possible sex-difference in the response was tested with a Mann-Whitney U-test (Dytham, 1999).

5.2.3.4 Influence of the male marking substance

In another experimental series a host plant (*C. arvense*), which had been previously marked by male *U. cardui* was tested against an unmarked plant. Marking of the host plant was performed immediately before the experiment in little observation cages (see chapter 3.2) for approximately two hours. The males were observed to ensure the presence of extensive marking on the plants. Thistles were then used immediately for two experimental runs (=1h) in the wind tunnel. Preliminary experiments showed, that effects of male marking declined after two hours. The plants were presented on the left side of the walking table as in the positive control of the combined experiments. 81 males and 93 females were tested. The preference for one of the plants was analysed via Wilcoxon Tests (Dytham, 1999) in SPSS (10.0 SPSS Inc.).

5.3 Results

5.3.1 Morphological characters of thistles selected for oviposition

Of the 218 thistles presented, 102 were galled, of 71 of them adult flies emerged. The height of the thistles analysed was normally distributed (K-S test: Z=1.272, p=0.079). In the mean the plants were 35.82cm (±20.66) high. The number of branches and the number of flower buds were not normally distributed and not transformable (K-S test$_{\text{branching}}$: Z=1.705, p=0.006; K-S test $_{\text{flower buds}}$: Z=3.702, p<0.001). The median of the branching number was 18.5 (quartiles: 4-25), the median of flower bud numbers was 0 flower buds (quartiles: 0-7).

The different classes of thistle characteristics (Table 5.1) were not equally present in the tested thistles. Table 5.3 gives an overview of the numbers of thistles in each class, the expected values (the amount of galled thistles resulting of proportional distribution) and the amount of galled thistles found in each class.

In the height classes there was no significant difference between the observed and the expected values (chi-square-test: χ^2=2.827, n=102, df=2, p=0.243); obviously the females did not prefer thistles of a specific height (Fig. 5.6). In contrast, females preferred *C. arvense* with a moderate branching level (chi-square test: χ^2=9.064, n=102, df=2, p=0.011, Fig. 5.7). They avoided plants with only weak branching. Females distinctly preferred *C. arvense* plants with no or few flower buds (chi-square-test: χ^2=11.136, n=102, df=2, p=0.004, Fig. 5.8).

Table 5.3: Numbers of thistles in the experimental population, expected values for galled plants and number of galled plants in each of the classes. Additionally the results of the chi-square tests are summarized.

	height [cm] $\chi^2_{(2)}$=2.868, p=0.24			branching $\chi^2_{(2)}$=9.082, **p=0.01**			flower bud number $\chi^2_{(2)}$=11.074, **p=0.004**		
class	small	middle	large	weak	moderate	strong	few	some	many
n thistles offered	87	59	72	96	49	73	169	27	22
expected values	40.63	27.64	33.72	44.93	22.92	34.15	79.03	12.66	10.29
n thistles with galls	35	35	32	33	35	34	93	6	3

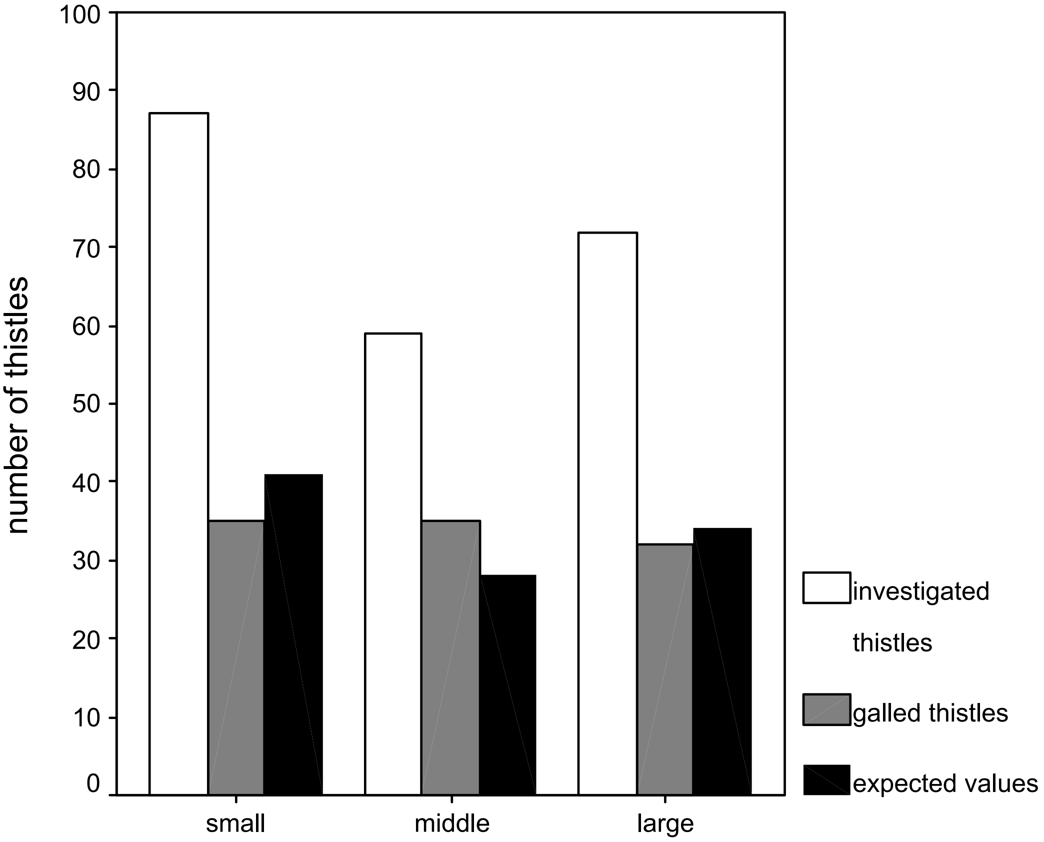

Fig. 5.6: Number of galled thistles, thistles in the experimental population and expected values for thistles galled belonging to the different height classes (n=102). Y-axis: number of thistles.

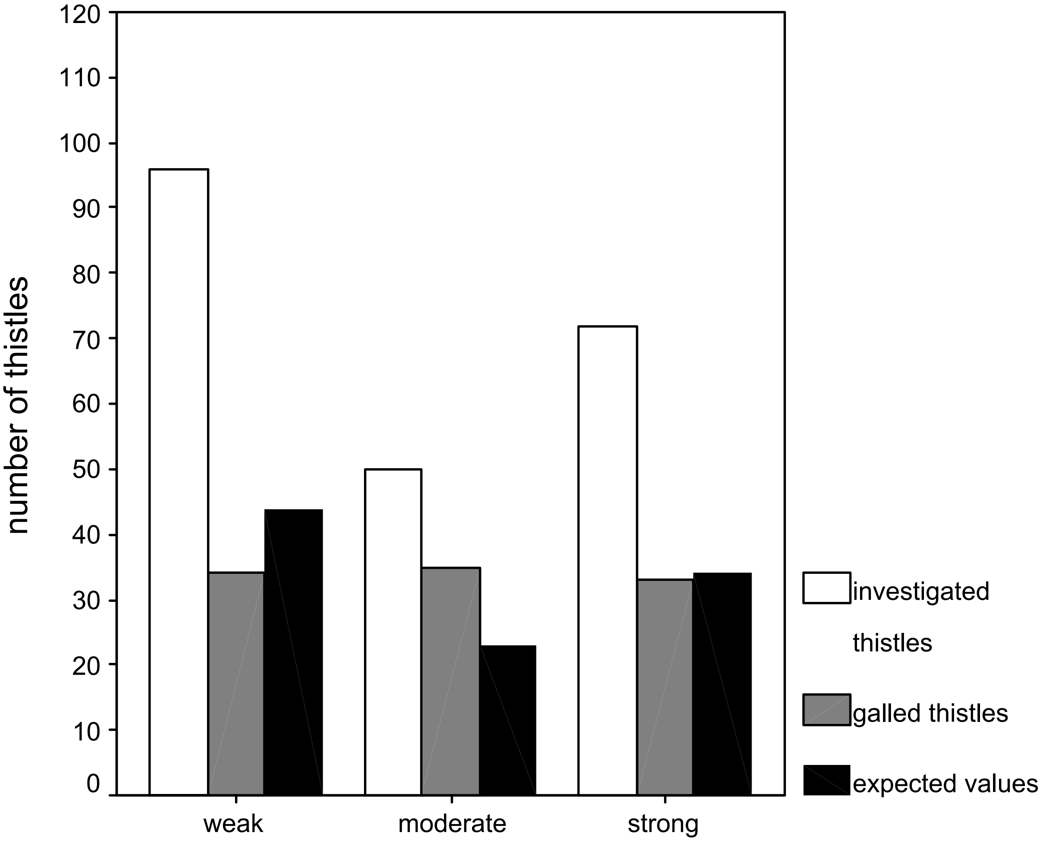

Fig. 5.7: Comparison of galled thistles, thistles in the experimental population and expected values for thistles galled (n=102). Y-axis: number of thistles belonging to different branching classes.

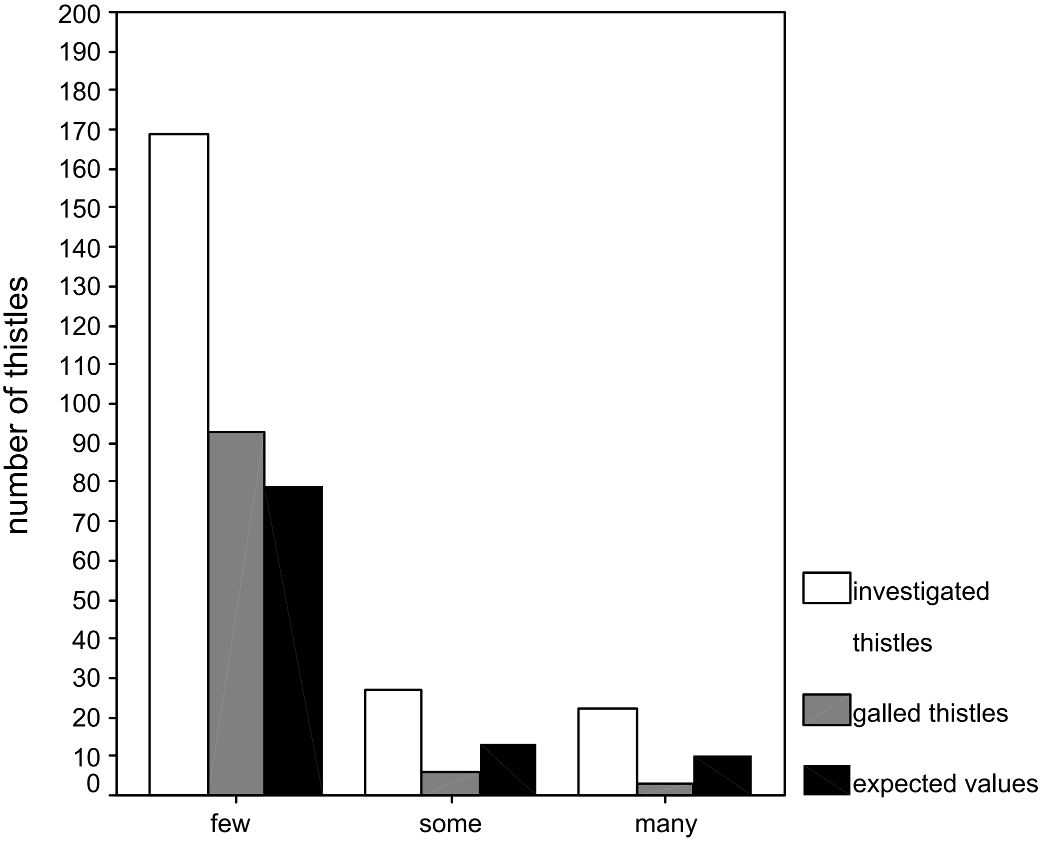

Fig. 5.8: Comparison of galled thistles, thistles in the experimental population and expected values for thistles galled in the different flower bud classes (n=102). Y-axis: number of thistles belonging to different bud classes.

5.3.2 Ability to distinguish between a host and a non host plant

83 females and 62 males were tested in the thistle recognition set-up: 34 females and 27 males showed a response. The numbers of responding flies in the different experiments were not normally distributed (K-S test: Z=1.487, n=35 (experiments, both sexes were pooled), df=1, p=0.024). Other experiments (see chapter 3) indicated that this may be due to individual variability. A comparison of the response of males and females towards host and non-host plant is shown in Fig. 5.9. The males significantly preferred the host plant against the non-host plant (Wilcoxon test: Z=-2.41, n=11 (experiments), df=1, p=0.019). The preference for the host to the non-host plant was even more prominent in the females (Wilcoxon test: Z=-3.4, n=17 (experiments), df=1, p=0.001), due to a higher sample size. Thus both sexes discriminate between a host and a non-host plant before they land on one of the plants.

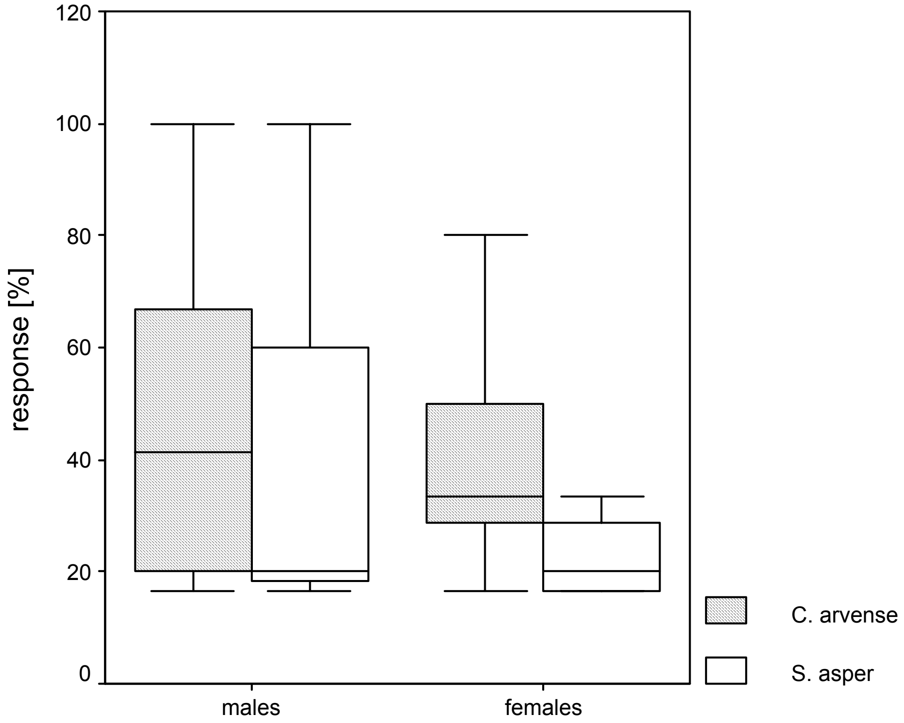

Fig. 5.9: Comparison of the response of males and females of *U. cardui* to a host plant and a non host plant. Y-axis: response in percent, lines in the boxes=median; boxes=25-75%, whiskers=minimum and maximum response.

5.3.3 Visual and olfactory detection versus positive control

102 females and 86 males were tested in the wind tunnel in the positive control set-up (Table 5.2): 29 females (28%) and 17 males (20%) showed a response (compare to the response ratios in chapter 4.3.1). In the visual set-up 117 females and 44 males were tested. 27 females and 10 males responded, which is 23%, respectively 22%. In the olfactorial set-up and the corresponding negative control 112 females and 53 males were tested. Especially the females responded also in the negative control. Of 39 females only 7 responded in the set-up with a plant alone, the others simply landed on the gauze curtain. These individuals were omitted from the graphs and analyses and only this corrected number of females is given. Of 22 males, which responded in the experiment, only two showed the same preference for the gauze curtain as the females. Thus in all the following analyses and graphs 20 males are counted as reacting positively. Fig. 5.10 shows a comparison between the response of males and females in the different set-ups.

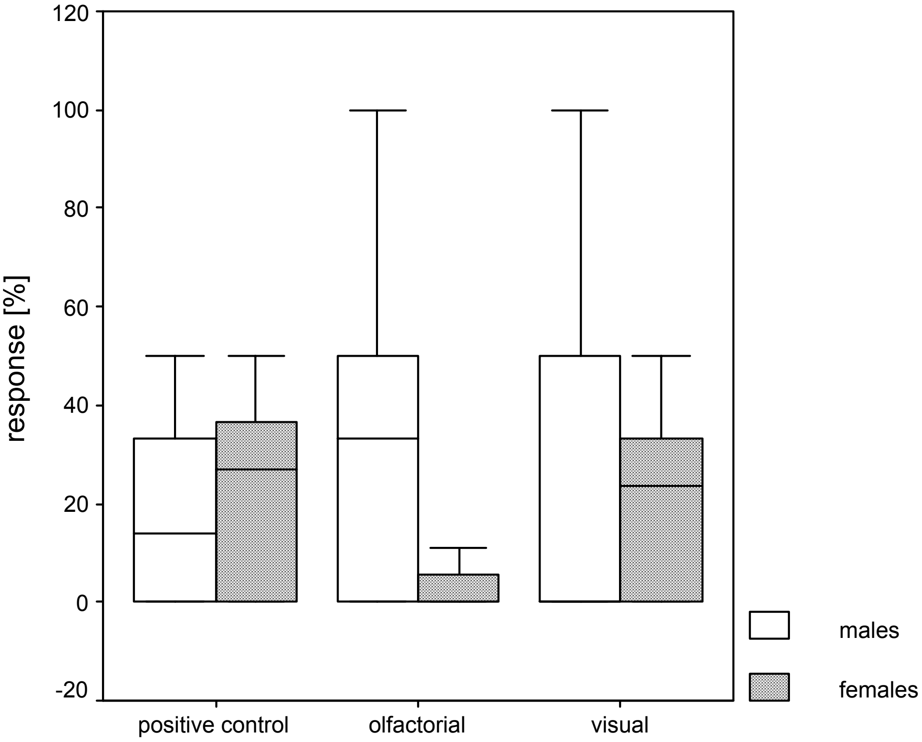

Fig. 5.10: Comparison of the percentage of responding individuals in the different experimental set-ups. Lines in the boxes=median; boxes=25-75%; whiskers=minimum and maximum percentage of response. The number of individuals responding in the negative control are already excluded (see above).

In order to analyse differences between the responses of individual flies in the different set-ups a Friedmann-Anova was performed for each sex. For the 102 females, which were tested in all treatments a significant difference resulted ($F_{(2,102)}=29.27$, $p<0.001$). A following Wilcoxon-Wilcox post-hoc test showed, that response in the olfactorial design (corrected by the negative control) is responsible for this difference (D=34.5, p=0.05). No statistical difference could be found for the 44 males, which passed all set-ups ($F_{(2,44)}=3.37$, p=0.185).

5.3.4 Additional olfactorial experiments

In 9 experiments, 13 of 34 males and 2 of 32 females responded to thistle odour. No male and no female responded in any of the negative control experiments. For the males, the difference between the response in the experimental set-up and the control set-up was statistically significant (Wilcoxon test: Z=-2.598, n=9 (experiments), df=1, p=0.009); in the case of the females there was no significant difference of the response in the two different treatments (Wilcoxon test: Z=-1.414, n=9 (experiments), df=1, p=0.157). There was a significant difference between the response of males and females, the males responded to host plant odour, while the females did not (Mann-Whitney-test: U=12, n=23, p=0.001, Fig. 5.11).

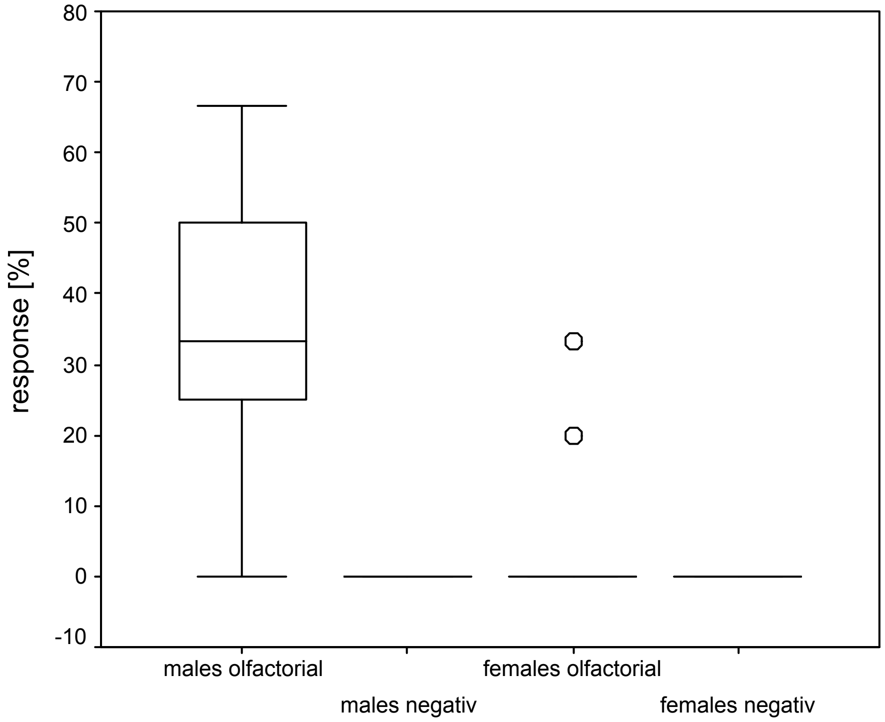

Fig. 5.11: Comparison of the response [%] of both sexes in experiments and negative control. Lines in the boxes=median; boxes=25-75%; whiskers=minimum and maximum response, open circles: outliers (1.5-3times inner-quartile distance).

5.3.5 Influence of the marking substance of the males

93 females and 81 males were tested if they preferred a thistle, which had been previously marked by a conspecific male: 25 males (30.9%) and 38 females (40.9%) responded in this set-up. The distribution of the reacting animals on male-marked respectively unmarked thistle is shown in Fig. 5.12. Males and females both significantly preferred marked host plants. In the case of the males the differences were not as prominent (Wilcoxon test: Z=-2.4, n=15 (experiments), df=1, p=0.018) as in the case of the females (Wilcoxon test: Z=-2.5, n=20 (experiments), df=1, p=0.011). There was no difference between the response of the two sexes (Mann-Whitney-test: U=1.5, p=0.4).

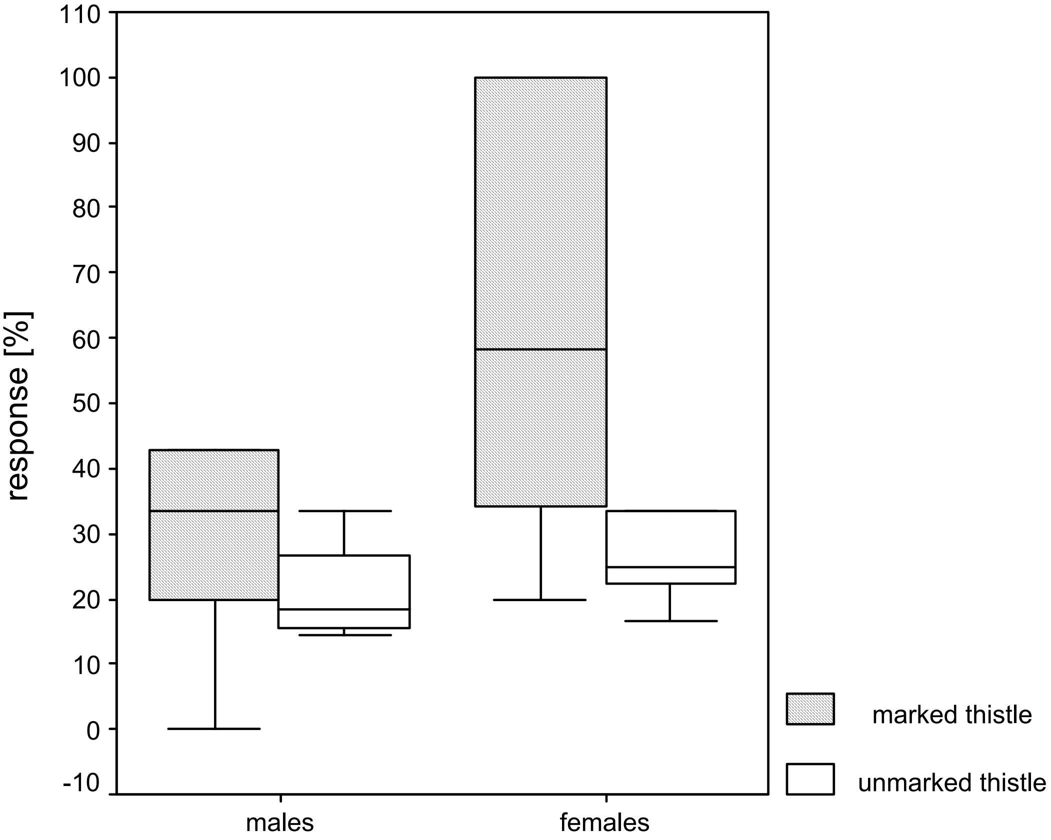

Fig. 5.12: Percentage of males and females which responded to the male-marked respectively unmarked thistle in the pheromone treatment. Lines in the boxes= median; boxes=25-75 %; whiskers=minimum and maximum response.

5.4 Discussion

Size (height or volume of the plant) of the host plant was apparently not an important cue of host plant suitability for females of *U. cardui*. A simple plant structure (few connections between plant parts) seemed to decrease the suitability of the host plant if only the amount of axillary buds (branching) was analysed. In contrast zero or few flower buds (simple structure) were preferable for female flies. Many flower buds indicate a higher developmental stage of the host plant, while the presence of many side shoots increases also the amount of suitable axillary buds. A high number of side shoots indicates a higher developmental stage of the plant, like the presence of many flower buds. If these results are compared to a study (Gingras *et al.*, 2002), which focused on the role of plant structure for host acceptance of a parasitoid (*Trichogramma evanescens* (Hymenoptera: Trichogrammatidae)), the complex influence of plant structure on host selection becomes apparent. In *T. evanescens* the host finding success of the parasitoid was higher on a plant with an overall simple structure. This effect could be explained by the connectivity (abundance of connections) and the heterogeneity (abundance and diversity of plant parts) of plant parts. In the case of *U. cardui* simple plant structure was only preferred if the number of flower buds was taken into account alone. If the host plant selection is regarded in relation to the number of side shoots a moderate amount was chosen. Here the influence of different aspects of plant structure seemed to be more complicated than in the case of *T. evanescens*.

Both sexes are able to discriminate between a host plant and a different species, which is common in the habitat of *U. cardui* before they select a plant. This suggests that the flies are able to recognise their host plant at a distance of 0.8m (see also chapter 5) For tephritid fruit flies Roitberg (1985) reports a reactive distance of 17cm to fruit clusters in trees. The responding distance reported for the apple maggot fly, *Rhagoletis pomonella,* to its host plant (not to fruit clusters on it) is maximally 2.5m, though the most frequent response occurred at a distance of 0.5m (Green *et al.*, 1994). But thistles are smaller than apple trees, thus *U. cardui* seems to have a longer responding distance compared to other fruit flies.

Experiments with a wind tunnel allowed the analysis of the usage of different senses during host plant recognition and selection under controlled environmental conditions, which were identical in the experiments performed. Wind tunnels are commonly used for experiments with moths, butterflies, beetles and some other insects (Bäckmann *et al.*, 2001; Larsson *et al.*, 2003; Linn & Roelofs, 1989 and references therein; Löfstedt *et al.*, 1989; Rojas, 1999; Sanders, 1986; Stensmyr *et al.*, 2003). Its use in dipterans is rare since flies are difficult to treat in a wind tunnel (C. Linn pers. comm.); so far it has been only employed for experiments with *R. pomonella* (Aluja & Prokopy, 1993; Fein *et al.*, 1982; Zhang *et al.*, 1999). Optimal light conditions and testing of several individuals simultaneously increased response of the flies as preliminary experiments indicated. Selection of a suitable wind speed did also influence the results positively (Mitchell, 1988; Zhang *et al.*, 1999).

Preliminary experiments hinted that the flies responded better if only the first half of the wind tunnel was used. This indicates that the odour plume farther from the source might already have been unfavourable for the flies or that the substances in it were present in very low concentration. Generally odour signals consist of short pulses of varying intensity (Murlis, 1992). Three different levels of odour structure can be separated (Murlis, 1992): 1) the large scale structure, which includes the shape and the average odour strength, 2) the small scale structure, which describes the fluctuating odour concentrations within a plume, which affect the input to the insects´ nervous system and hence their response to it, and 3) the time-average structure, which determines the probability that an insect will contact the odour plume at different locations downwind of the source. The farther away an insect is from the odour source, the weaker the signal pulses are on average (Murlis, 1992). Nearer to the source the peak-to-mean ratio is considerably reduced but the rate, at which odour concentrations rise at the leading edge of bursts is increased. The latter situation was present in the wind tunnel. Measurement of the wind speed in different edges of the tunnel showed a nearly constant wind speed of 0.55m/s. But, as Murlis (1992) summarizes, the fine scale structure of odour plumes varies with time, which could not be analysed in the above mentioned measurements. The habitat structure will have a major impact on the odour plume, therefore it is difficult to transfer results of wind tunnel experiments to nature (Kramer, 1986; Payne *et al.,* 1986).

Only few studies analyse characteristic volatiles of plants of the genus *Cirsium*. Several *Cirsium* species contain numerous flavones and polyacetylenes (Christensen, 1992), but all these studies have only analysed flowering plants (for example Christensen, 1992; Ibarra, 2002). *U. cardui* preferred plants with zero or few flower buds. Thus the volatile profile perceived consisted only of green leaf volatiles. The response of the male flies in the wind tunnel indicated that these volatiles were sufficient for host plant selection. Nevertheless for a final corroboration of the underlying physiological processes the performance of electrophysiological experiments is necessary. Some preliminary investigations were performed at the Institute for Forest Zoology and Forest Conservation of the Georg-August University Göttingen. In these the response of a female antenna to the olfactory profile of a natural host plant and of a marked host plant was tested. Additionally the response of two different male antennae to the olfactory profile of a natural host plant was analysed (see below).

Males of *U. cardui* can recognise their host plant on olfactory *or* on visual cues. The selection process is not influenced by the usage of one of these two types of sensory information. In contrast, the females fail to recognise their host plant, if only olfactory cues of it are available. They were thus able to recognise the host plant using visual cues alone. The different response to olfactory cues indicates a physiological difference between males and females of *U. cardui.* Additional electrophysiological experiments in Göttingen seemed to corroborate this suggestion. The antennae of the males were stimulated upon perception of the olfactory profile of *C. arvense*, while those of the females were not. Similar studies which analyse the perception of host stimuli of both sexes are rare (Agee *et al.*, 1982).

In *U. cardui* both sexes responded similar to visual cues of their host plant. In a comparison of different polyphagous, oligophagous, and monophaghous dipteran species Prokopy & Owens (1978) found that many oligo- and monophagous species tend to be visual specialists, since (in many species) the visual aspects of the host plant structure could elicit a positive response, as shown for both sexes here. If the spectral sensitivities of three different tephritid pest species (*Ceratitis capitata, Dacus oleae* and *Rhagoletis cerasi*) are compared between each other and between the different sexes (Agee *et al.*, 1982), both sexes in all three species responded differently. It was not possible to determine which visual cue or cues elicited male and female response in the course of the present investigation, the hue (dominant wavelength) (Agee, 1985; Aluja & Prokopy, 1993; Green, 1986; Prokopy *et al.*, 1983a; Strom & Goyer, 2001), the intensity of reflected light (brightness) (Katsoyannos *et al.*, 1985), saturation (chroma or tint) of a colour (Katsoyannos *et al.*, 1985), general physical features like the shape (Green *et al.*, 1994; Moericke *et al.*, 1975; Prokopy *et al.*, 1983b; Schmidt, 1994), the contrast of the provided cues to the background (Katsoyannos *et al.*, 1986 and references within), or several of these stimuli together (Prokopy, 1986; Roitberg, 1985).

Generally, it is true for nearly all species for which olfactory perception of host plant cues has been analysed, that they react to blends of volatiles and only rarely to single components (Bäckmann *et al.*, 2001; Ignacimuthu *et al.*, 2000; Linn & Roelofs, 1989; Nielsen, 1990; Tollsten & Bergström, 1988). The influence of green leaf volatiles compared to other olfactory cues of the host plant on host plant selection of herbivorous insects has been discussed by a number of authors (Green *et al.*, 1994; Mitchell, 1988; Müller & Hilker, 2000). In *U. cardui* green leaf volatiles seem to act directly as attractants, at least for the males. It is known, that the amount of green leaf volatiles varies between plant species and even within one species depending on developmental stage, temperature and potential damage by other herbivores (Schoonhoven, 1990). These effects need to be analysed (Bäckmann *et al.*, 2001). Developmentally induced changes in the volatile profile may be more important for herbivorous insects, which select for fruits (see for example Carle *et al.*, 1987; Haisch & Levinson, 1980). Females of the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) are stimulated to land more often, if they perceive green leaf volatiles (Warthen *et al.*, 1997). The response to male odour was also increased by green leaf volatiles (Warthen *et al.*, 1997). This may also apply to *U. cardui* females, but experiments are necessary. Often green leaf volatiles are not reported as direct host plant attractants. For example the larvae of the oligophagous chrysomelid *Cassida denticollis* (Coleoptera: Chrysomelidae) are not directly attracted by green leaf volatiles, but their ability to discriminate between a host and a non-host plant is enhanced (Müller & Hilker, 2000). Chemical information of the host plant may still be important for females of *U. cardui*, but only after landing. Perception of epicuticular waxes may for example influence the behaviour on a plant selected for oviposition (Eigenbrode & Pillai, 1998; Grant *et al.*, 2000; Kombargi *et al.*, 1998) and also the perception of low-range volatiles of plant organs (Eisemann & Rice, 1985). Once on the plant the *U. cardui* female may use the volatile information of the plant, but not during selection from a distance, as shown in the wind tunnel experiments.

Interaction of two different senses in such a complex process like host plant selection is quite common (Aluja & Prokopy, 1993; Beehler *et al.*, 1993; Green *et al.*, 1994; Pierce & Elgar, 1985). In *U. cardui* this seemed to apply only for the males if volatile information of the host plant was analysed alone (see below). Often authors differentiate between synergistic, additive or hierarchical usage of the interacting senses (Fischer *et al.*, 2001; Harris & Miller, 1988). But in the males of *U. cardui* none of these three possible effects could be observed, since the males perform equally well with only one of the two senses, which is remarkable.

Both sexes significantly preferred plants, which were previously marked by conspecific males. This discrimination can only be performed according to olfactory cues, since the plants presented were of the same height and had the same number of axillary and flower buds. Male preference for plants marked by conspecifics may indicate, that the costs of a fight with a conspecific male are lower than the risk of a deadly encounter with a spider. Unfortunately there are only few studies concentrating on the aspects of male territorial fights (Fregin, 2003; Marden & Rollins, 1994; Papaj, 1994; Strohm & Lechner, 2000; Yuval *et al.*, 1994) and I know of none that actually measured the costs of fighting. For the male, which has already selected for a territory and marked the plant, there are positive as well as negative effects of the marking. On the one hand it is now able to attract females and reproduce; on the other hand it also attracts opponents with whom it has to fight. But these costs are taken into account in order to get access to mating partners. In contrast, the effect of the marking substance for the females is only beneficial. The marking substance of the males signals the presence of a mating partner to the female and additionally a spider-free plant. The latter effect may be even more important than the presence of a mating partner, since the females spent very much time on probing and oviposition on a plant (chapter 3). This is only possible on an enemy-free plant.

Many fruit fly species possess a male sex pheromone, which is produced in the gland complex of the rectal sac (Baker *et al.*, 1990; Jacobson *et al.*, 1973; Robacker *et al.*, 1985). It is assumed, that these pheromones are species specific and thus ensure intraspecific mating (Fletcher & Giannakakis, 1973; Jang *et al.*, 1994; Kobayashi *et al.*, 1978). Like the volatile profile of host plants, most insect semiochemicals consist of several components (Hölldobler, 1999), which together induce a better response than the single components (Jang *et al.*, 1994; Larsson *et al.*, 2003). This does not apply to the marking substance of *U. cardui* males. The only substance, which has been found in the respective gland was 4-methyl-3*Z*,5-hexadienoic acid (Frenzel *et al.*, 1990), in other fruit flies always a mixture of substances could be found (Jacobson *et al.*, 1973). After isolation and identification of this substance, its effect on females of *U. cardui* was tested (Frenzel *et al.*, 1990), since it was assumed, that they should prefer marked plants. But the opposite was found, while in the wind tunnel experiments presented here the positive effect of the marking substance was significantly. In the study by Frenzel *et al.* (1990) only the effect of the pure hexadienoic acid was tested. Thus the avoidance of the females in those experiments may be due to concentration effects (see for example Matsumoto, 1970). There exist various studies, in which testing of pure pheromones or pheromone blends failed, while tests using naturally marked host plant material, respectively live males were successful (Heath *et al.*, 1999; Howse *et al.*, 1986; Kobayashi *et al.*, 1978). To avoid these concentration difficulties naturally marked plants were used here. In additional electrophysiological experiments, which were performed in Göttingen, also naturally marked plants were employed and indeed a response from female antennae was observed.

The intrinsic state of the female presumably has an impact on their response to the marking substance of the males (Robacker *et al.*, 1985). Directly after mating females usually are unresponsive to male sex pheromones, since the search for a suitable oviposition place becomes more important (Robacker *et al.*, 1985). After egg deposition the responsiveness should increase again, since *U. cardui* females depend on frequent mating in order to ensure fecundity (Freese & Zwölfer, 1996). These effects were not tested in the wind tunnel, but preliminary behavioural observations with *U. cardui* females in different mating state suggested that these effects of intrinsic state of the females apply in *U. cardui*, too.

The results presented show that the selection for territories by the males is important since male selection for territories precedes female selection for oviposition sites and has a major impact on it. The females seemed to be totally unreceptive for the olfactory profile of their host plant, but plants marked by conspecific males were readily distinguished according to their olfactory profile. It is likely that they search for marked plants in their natural environment and only accept unmarked thistles as oviposition places when no marked ones are present. In their search for suitable oviposition sites the females thus depend on an optimal preselection of the males. Specialised herbivorous insects where the larval host plant is also the rendezvous-place of the adults and the territory of the males may include a similar selection behaviour of the males preceding female selection for oviposition sites.

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6 Morphology of the ovipositor of the thistle-stem gall fly *Urophora cardui* (Diptera: Tephritidae)

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The ovipositor of the specialised gall fly *Urophora cardui* consists of three different parts, of which usually only the oviscape is visible. The three parts have different structures and therefore different functions. The oviscape has a hard, cuticular structure comparable to the integument of the abdomen, but lacking the very small covering setae of the latter. The oviscape protects the other two parts of the ovipositor. The soft eversible middle part of the ovipositor has a rasp-like cuticular structure that facilitates the penetration of the ovipositor into the host plant tissue. By its flexibility it also moves and turns the aculeus, the rigid tip of the ovipositor. The aculeus has a smooth surface, in contrast to the two basal parts of the ovipositor. The aculeus bears different sensillae, which serve to examine possible oviposition sites. Additionally the aculeus places the eggs into suitable axillary buds of young lateral side shoots of *Cirsium arvense*.

At least three morphologically different sensillae types could be identified on the ovipositor of *U. cardui*. These were setae on the oviscape, which may have mainly mechanosensory function, and uniporous pegs and campaniform sensillae on the aculeus. Possible functions in the highly specialized gall-former *U. cardui* may be the examination of the tissue of axillary buds of *C. arvense* and the reception of chemical signals of the buds including signals indicating its water content. Most of the sensillae on the aculeus are arranged in a regular sensory field apical of a groove, which allows a selective placement of the eggs.

6.1 Introduction

Fruit flies (Tephritidae), as well as other insect taxa with endophytic larvae, are known for their highly developed host-plant recognition capability and discrimination of suitable oviposition sites (Drew & Lloyd, 1987; Fletcher & Prokopy, 1991; Prokopy & Roitberg, 1989; Prokopy & Bush, 1973). Crucial factors for the evolution and radiation of fruit flies were a) the usage of the inner plant tissues as oviposition substrates and b) the evolution of a toughened aculeus to allow penetration of plant tissue (Diaz-Fleischer *et al.*, 1999). Many of the tephritid species associated with Asteraceae as host plants are well-adapted to exploit one or a small number of closely related host-plant species (Aluja & Norrbom, 1999; Diaz-Fleischer *et al.*, 1999): the structure of their ovipositor is specialised to the characteristic features of their host plant (Diaz-Fleischer *et al.*, 1999). If, for example, the host plant epidermis is thick, the aculeus tip is more pointed, and the tip angles are more acute to allow positioning of the eggs (Diaz-Fleischer *et al.*, 1999). As the ovipositor of insects with endophytic oviposition (galling and mining species, endophytic parasitoids), is the only part of the body in direct contact with the specific oviposition site, it functions not only as a tool for oviposition, but must also function as a finely-tuned perception organ, determining the quality of the interior tissues of the host (Preszler & Price, 1988; Price, 1990; Pyke, 1984). In fruit flies the evolution of the highly sensitive ovipositor is considered as one key innovation factor in the evolution of a fine-tuned selection behaviour on an already chosen host plant (Diaz-Fleischer *et al.*, 1999).

Sensillae on the ovipositor and the antennae enable tephritids to distinguish between host-plants that are already occupied by conspecific larvae and those still vacant (van Lenteren, 1972). The ovipositor provides the ultimate information, which is decisive for the process of oviposition. Like the antennae it contains different types of sensillae, which produce different signal types (Brown & Anderson, 1998; Le Ralec & Wajnberg, 1990). Generally the responsiveness of sensillae on the antennae depends on the sex, age and experience of the insect (Prokopy *et al.*, 1991; Rausher & Papaj, 1983; Zacharuk, 1980). Thus it can be assumed, that the responsiveness of the sensillae on the ovipositor are also influenced by the age and experience of the females. The physiological and ultrastructural features of sensillae, which allow their specialisation are still unknown (Zacharuk, 1980).

Sensillae can be classified according to their function or their morphology (Altner & Prillinger, 1980). The function of a sensillum is determined by its enervation (Osborne, 1970). Most common are mechano- and chemosensillae, additionally there occur hygro- and thermosensillae. All these types are primary sensory cells (Altner & Prillinger, 1980). More important for these investigation are different morphological types. In nearly every morphological study different names are used for similar structures. This is partly due to the fact, that until the 1980s it was assumed, that the morphology and the function of a sensillum are linked (Altner *et al.*, 1977), which is not the case (Zacharuk, 1980). Fig. 6.1 summarizes the sensillum types and the descriptions used here. Hair-like structures of varying length are referred to as setae. Campaniform sensillae or simply pits resemble a groove with a small dome in the middle. A synonymous name is sensillum basiconica. Very small setae with a blunt tip, also inserted in a groove appear with the description sensillum coeloconica or simply pegs.

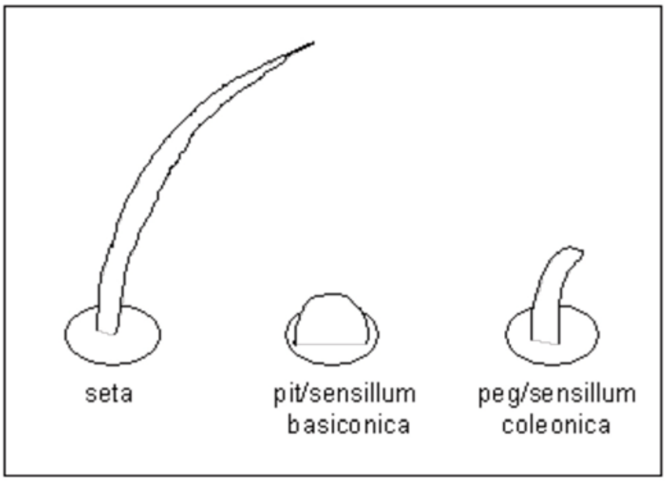

Fig. 6.1 : Scheme of the morphologically different sensillum types in the present study (after (Brown & Anderson, 1998; Hooper *et al.*, 1972; Le Ralec & Wajnberg, 1990; McIver, 1975; Rice, 1976) and (Schneider & Steinbrecht, 1968)).

To obtain information on the kind of sensillae and therefore the mechanisms of the selection for oviposition sites on an acceptable host plant the ultrastructure of the ovipositor of the thistle stem gall fly *Urophora cardui* (Diptera: Tephritidae) was examined.

In *U. cardui* the ovipositor has three main functions: 1) it is the mating organ, 2) the females probe the interior of the axillary buds of the host plant, *Cirsium arvense,* with the ovipositor-tip, and 3) using the ovipositor the eggs are deposited inside the tissue of the axillary bud. The extensive probing behaviour (see chapter 3) implies, that several sensillae may be present on the ovipositor.

6.2 Material and Methods

Ten adult females of *U. cardui*, which had died naturally in the rearing containers, were air dried for at least two weeks. All of them had been mated and had laid eggs for at least three days before dying. The oviscapes (=sclerotized ovipositor sheath (White, 1988)) of seven females were separated from the rest of the body with a sharp razorblade. Subsequently the inner parts of the ovipositor were gently pulled out of the oviscape until fully extended and fixed on a microscope slide with a double-sided glue strip. To obtain information on the position and orientation of the structures on the ovipositor, as well as on the mechanism of the ovipositor extension three females were mounted as whole individuals. Their aculeus (=piercing part of the ovipositor (White, 1988)) was either unextended or only slightly extended. All preparations were sputtered with a gold-argon mixture in a S150A Sputter coater, twice for one minute. The ovipositors were examined and photographed with an Environmental Scanning Electron Microscope (Philips XL30 ESEM, Philips, Hilversum, Netherlands) at 80 kV. A scale bar and the magnification are included on every photograph. The structures of the different ovipositor-parts were described following the nomenclature proposed by White (1988).

6.3 Results and Discussion

When the ovipositor of *Urophora cardui* females is not extended only the oviscape is visible (Fig. 6.2), allowing easy separation of males and females. The oviscape is approximately 1000µm long, has a basal diameter of approximately 500-600µm narrowing to approximately 200µm. The oviscape is covered with several setae of different length, like the abdomen.

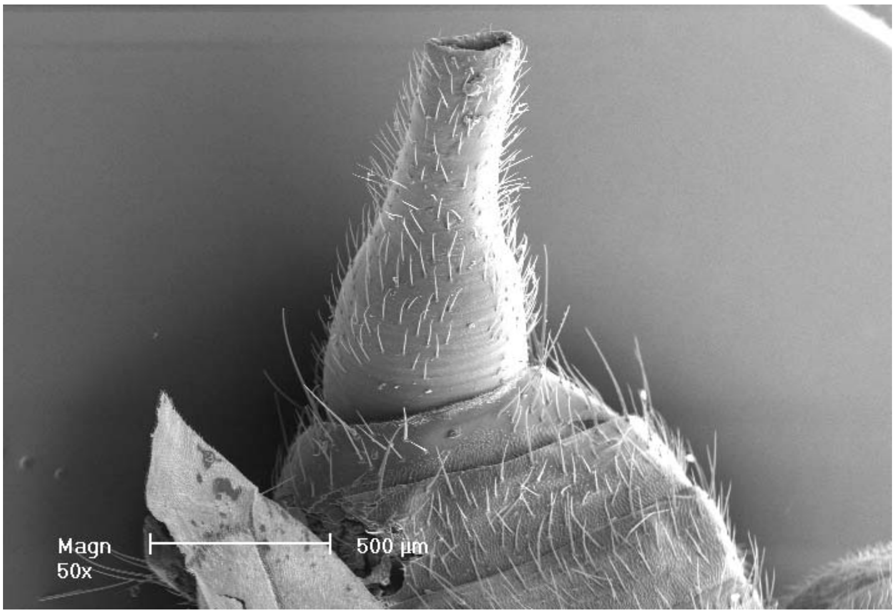

Fig. 6.2: Ventral view of the oviscape of an *U. cardui* female. In the lower part of the picture the last abdominal segments are visible. The plain structure on the left of the picture is a part of the wing.

The setae (Fig. 6.3) are inserted in a capsule with an elongated hourglass-shaped orifice, which facilitates movements in one plane (see McIver, 1975). They are not perfectly round in diameter, but distinctly fluted and have a sharp tip. The length of the setae on the oviscape ranges between 30-150µm, thus they are on average smaller than the setae on the abdomen (Fig. 6.2), which range from 10 to more than 800µm. Additionally the abdomen of *U. cardui* is densely covered with fine hairs (30µm and less), which are absent on the oviscape. In other studies of different insect species various types of setae were distinguished according to their size and their slightly different shape (for example: Gaffal & Hansen, 1972; Hooper *et al.*, 1972; Jones, 2000; Valencia & Rice, 1982). Generally such setae possess mechanosensory functions, but they can additionally be enervated by chemosensory neurons (McIver, 1975).

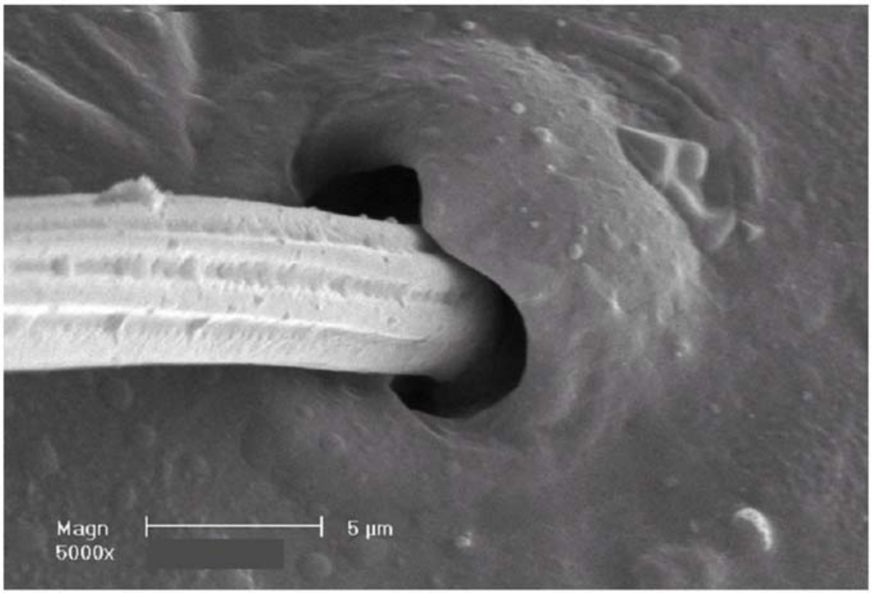

Fig. 6.3: Seta on the oviscape of an *U. cardui* female with its characteristic articulation, the hourglass-shaped orifice in which it is inserted in the cuticle of the oviscape.

Another remarkable attribute on the oviscape of *U. cardui* is one pair of round orifices (Fig. 6.4) on each side. Although no microscopic studies of the interior tissue of the ovipositor of *U. cardui* have been published, it is conceivable that the orifices are connected with glands. With a diameter of 25µm they appear to be too large to be a stigma (diameter of stigmata=0.5-17.5µm (Wehner & Gehring, 1995)).

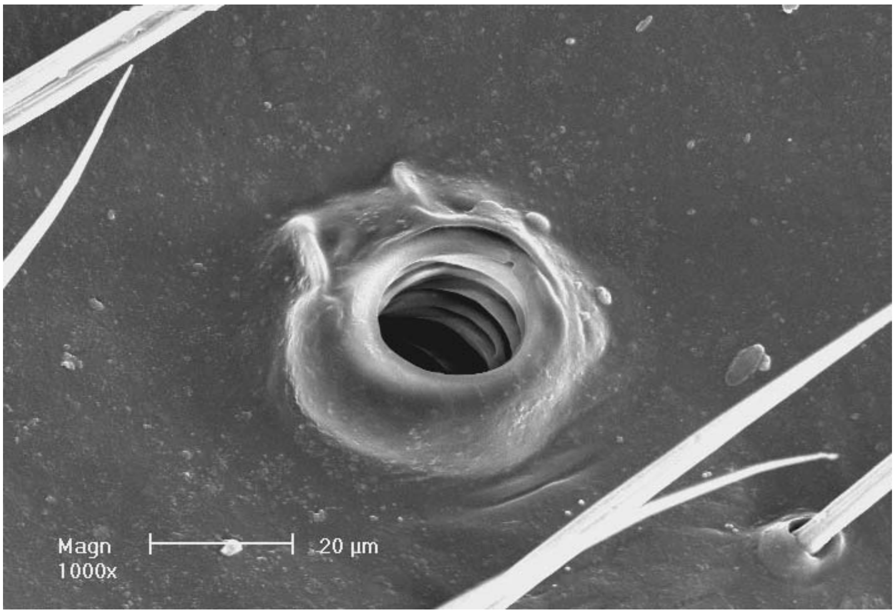

Fig. 6.4: One of the paired orifices on the side of the oviscape of an *U. cardui* female.

Usually the flexible eversible ovipositor sheath and its tip, the aculeus, are protected inside the hard oviscape (see Altner & Prillinger, 1980). The extension process of the ovipositor resembles the extension of the fingers of a glove and the inner parts are everted (Austin & Field, 1997). Fig. 6.5 shows the start of the extension. In all behavioural experiments described in this thesis the females only everted the ovipositor for cleaning, for probing a possible oviposition site and for oviposition (see chapter 3). In the last two cases the ovipositor was directly introduced into the plant tissue.

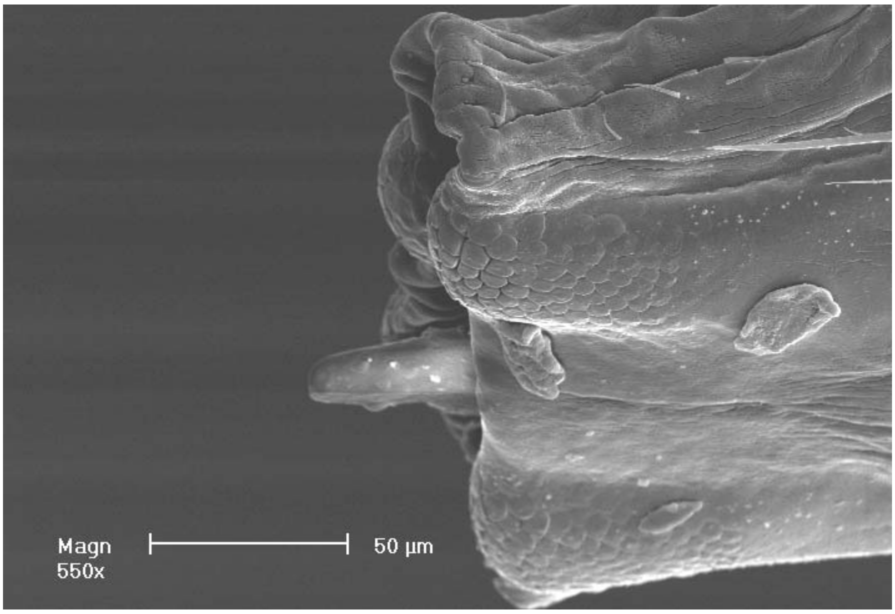

Fig. 6.5: Tip of the aculeus (left) and parts of the eversible ovipositor sheath (right) in an early phase of the extension of the entire ovipositor.

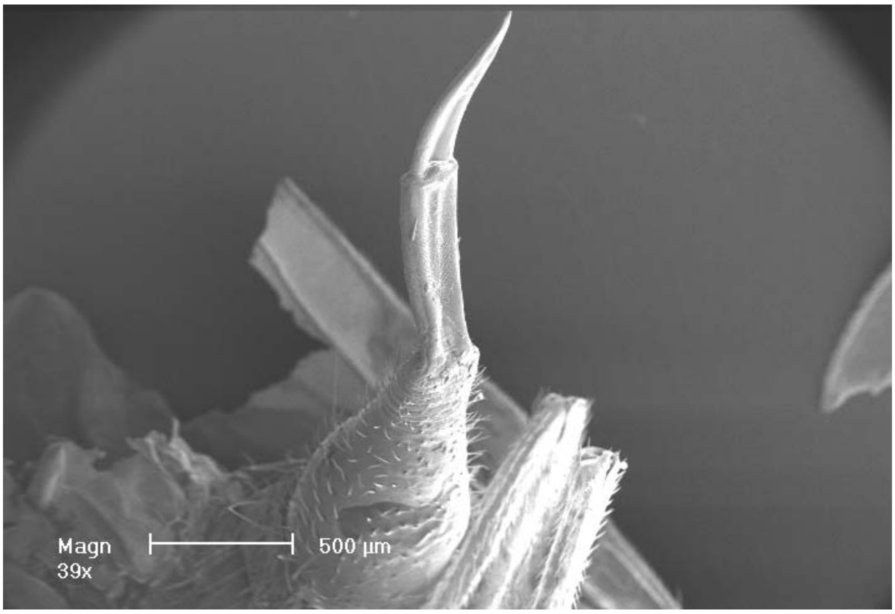

Fig. 6.6: Lateral view of the fully extended ovipositor of *U. cardui*, the upper part is the aculeus, below the middle part, the basal part of the ovipositor, the oviscape, can be seen next to the parts of the legs on the right (compare Fig. 6.2).

As shown in Fig. 6.6, the ovipositor of *U. cardui* consists of three parts: the previously described oviscape, the eversible, rasp-like middle part of the ovipositor sheath and the aculeus, which forms the tip of the ovipositor (Fig. 6.5, Fig. 6.6). This tripartite construction of the ovipositor is common in insects (for example: Austin & Field, 1997; Hooper *et al.*, 1972; Klass, 1997). The total length of the ovipositor of *U. cardui* is approximately 1800µm, with a basal diameter of 500-600µm, narrowing to 10.75µm at its distal end (see Hawke *et al.*, 1973).

The rasp-like structure (Fig. 6.7) of the middle part of the ovipositor facilitates the drilling of the ovipositor into the host plant tissue. In contrast to the oviscape and the aculeus no sensory organs were visible on its surface (Fig. 6.7). At the junction of the extensible middle part and the aculeus, the eggs leave the body of the female (Fig. 6.8). Here, a fine membrane forms a cover for the oviduct (see SEM photographs in Austin & Field, 1997). It may have a protective function (H. Zwölfer pers. comm.). Unfortunately, this membrane was partly destroyed in the preparation process, thus it is no longer in its usual arrangement in Fig. 6.8.

It is believed that generally in tripartite ovipositors the eggs are passed along the ovipositor by peristaltic waves. These are produced by the movement of internal valves, presumably supported by internal ctenidia or spines (Brown & Anderson, 1998).

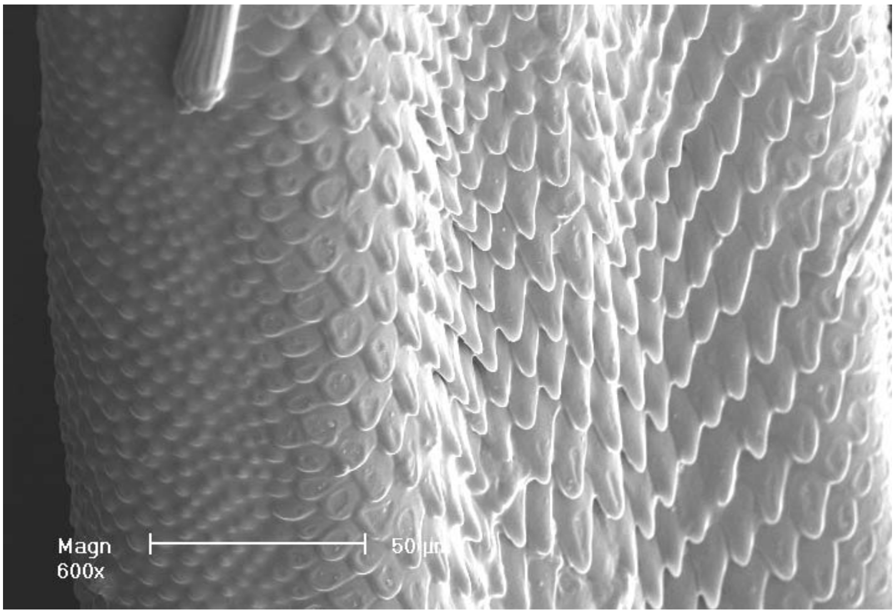

Fig. 6.7: The eversible ovipositor sheath shows a rasp-like surface-structure.

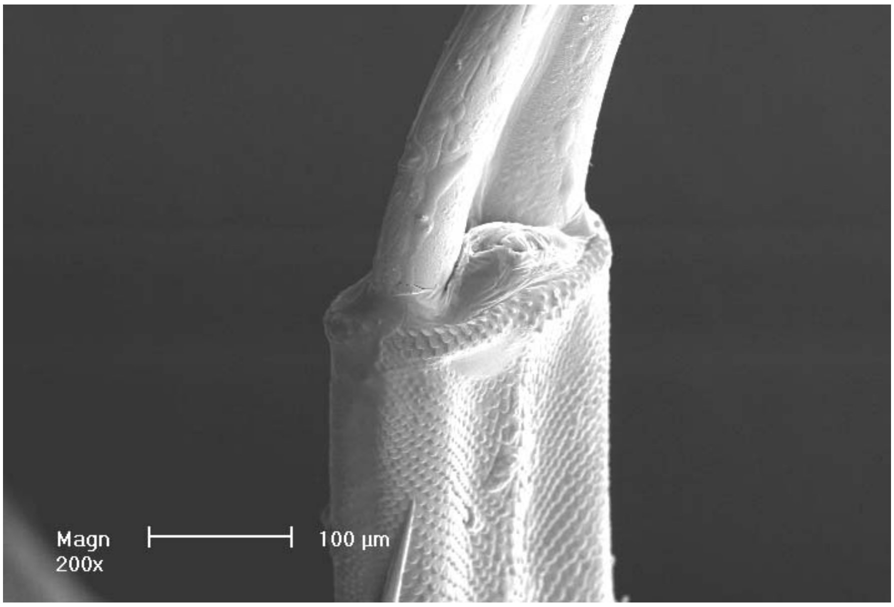

Fig. 6.8: Junction between the middle part of the ovipositor and the aculeus, the bulbous structure at the lower part of the aculeus above the rasp-like structure are the remainders of the membrane.

The ventral surface of the aculeus is smooth in contrast to the basal parts of the ovipositor (Fig. 6.9). In the middle of the aculeus there is a furrow of approximately 70µm width. After the egg has left the body of the female it glides along this furrow (compare for example with Austin & Browning, 1981; Klass, 1997; Scudder, 1961). This furrow ends in a groove, which presumably allows exact positioning of the egg inside the host plant tissue (compare to Zacharuk *et al.*, 1986). The smooth structure of the aculeus apparently provides protection of the eggs during the positioning–process. Fig. 6.10 depicts a close-up of the groove.

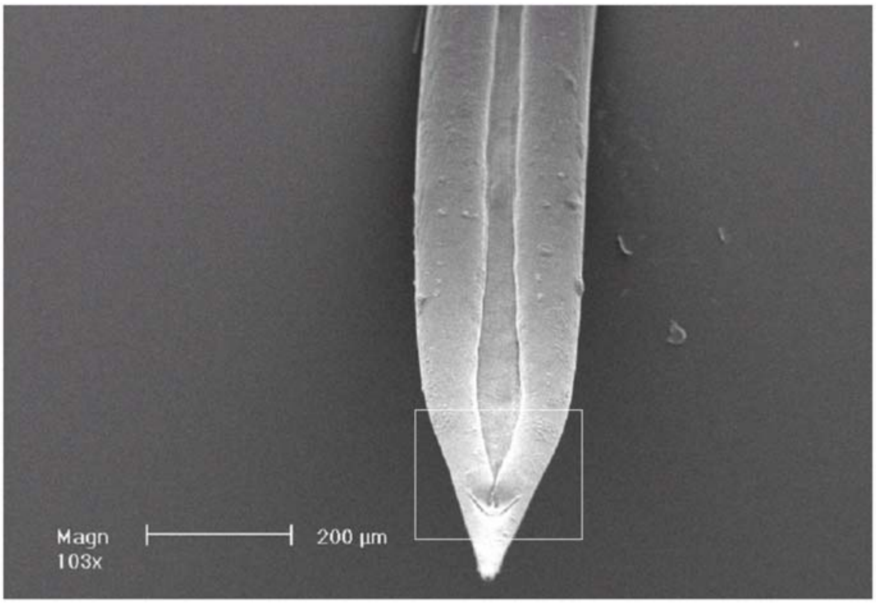

Fig. 6.9: Ventral view of the aculeus with the egg channel and the groove. The section of the picture contained in the box is further magnified in Fig. 6.10.

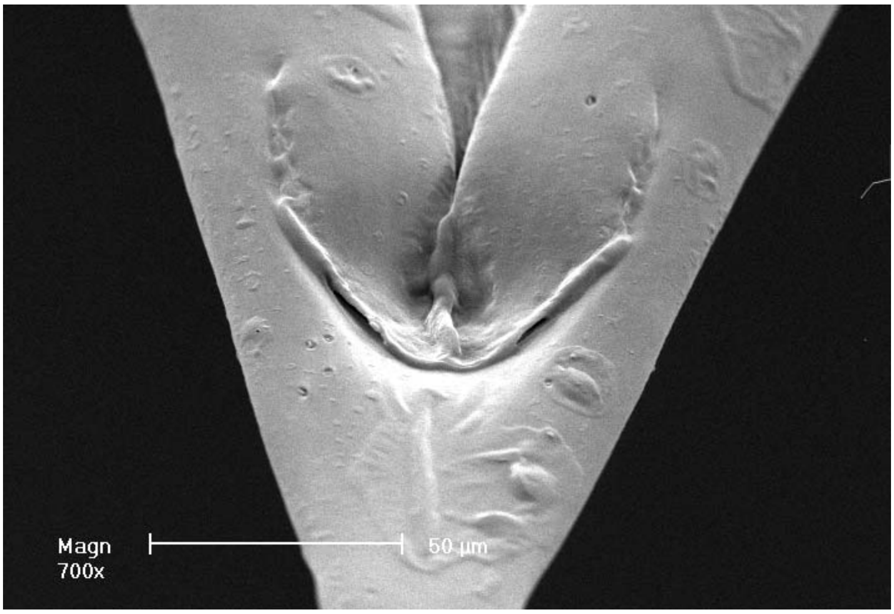

Fig. 6.10: Detail of the aculeus showing the groove, which may allow exact positioning of the egg in the host-plant tissue, while protecting it from damage.

The dorsal side of the aculeus is covered with nearly parallel slits of varying lengths (Fig. 6.11). Their arrangement is oblique-angled and points toward the middle of the aculeus. The function of these structures is yet unclear and similar structures in related species have not been reported. The arrangement of the slits and their size is reminiscent of the cuticular slit organ of spiders as described by Barth (1972).

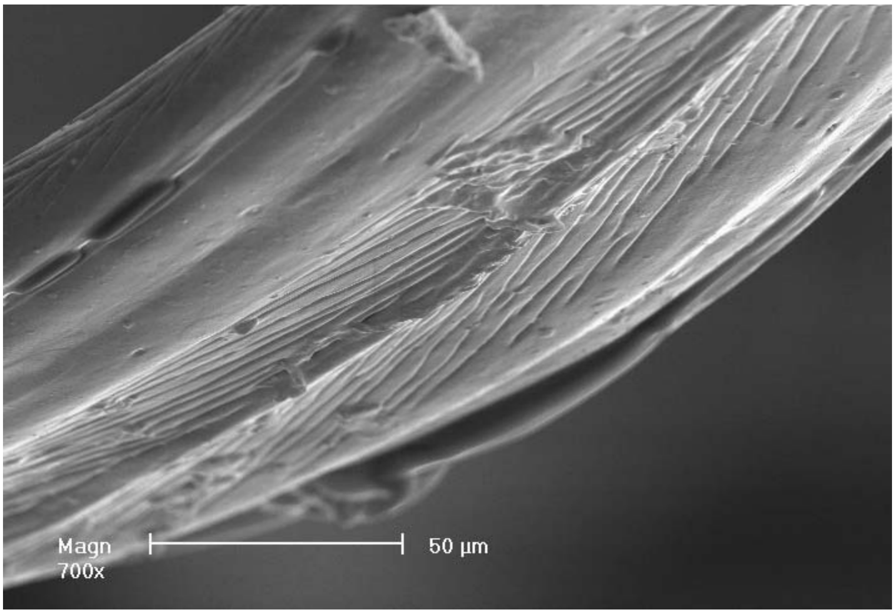

Fig. 6.11: Dorsal view of the aculeus with slits, which point towards the middle of the aculeus and are nearly parallel on each of the two sides.

On the aculeus no mechanosensory setae similar to those on the oviscape (Fig. 6.2) were observed. Some smaller sensillae were detected (Fig. 6.12). Most of them are concentrated apically of the groove (Fig. 6.13). The sensillae on the aculeus have presumably different functions than the setae on the oviscape. They are only used by the females if the ovipositor is inserted inside the host plant tissue. The setae on the oviscape, in contrast, are always exposed to the external environment and not only used for probing and oviposition behaviour.

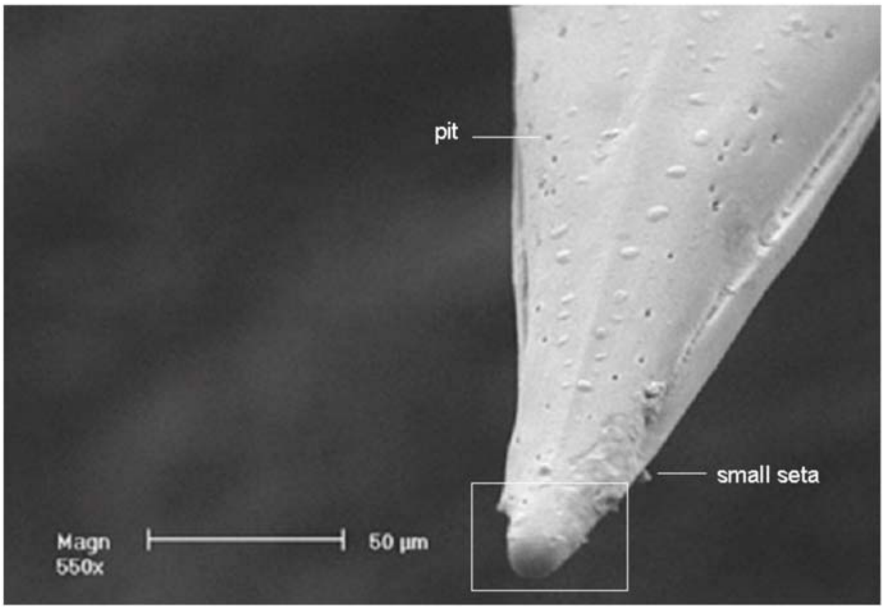

Fig. 6.12: The tip of the aculeus showing several pits and small setae. The section of the picture contained in the box is further magnified in Fig. 6.13.

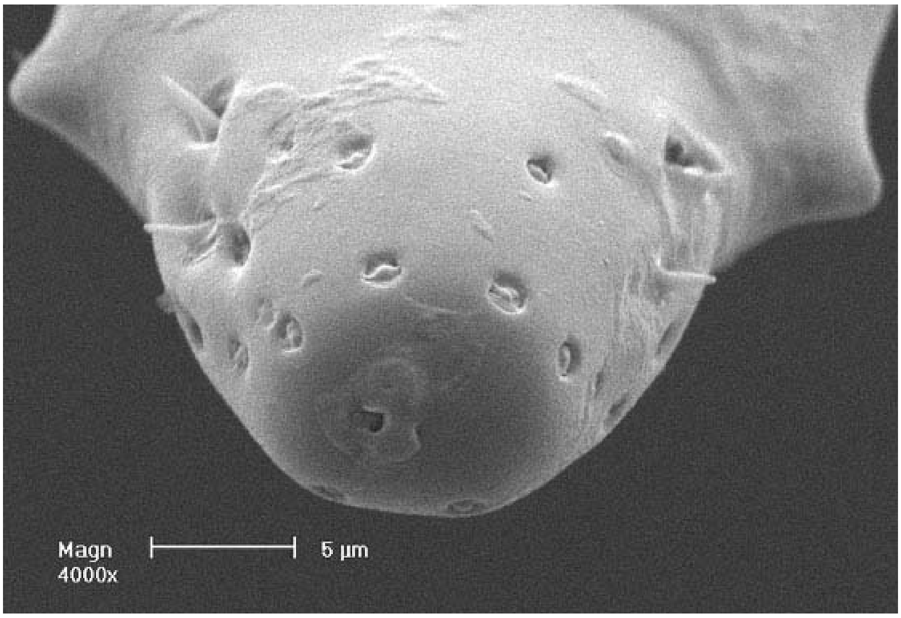

Fig. 6.13: Concentration of different sensillae types on the tip of the aculeus forming a sensory field.

The sensory field at the tip of the aculeus (Fig. 6.13 and Fig. 6.14) may enable the females of *U. cardui* to analyse the internal structure of the host plant tissues. It is impossible to assign the sensillae to different functions, since there exist no TEM studies of the innervation of the different sensillae. According to the SEM and TEM studies performed by Altner (1977) and Schneider & Steinbrecht (1968) the sensillae may belong to the types sensilla basiconica and sensilla coeloconica. Both are chemosensory sensillae, which have additional mechanosensory functions.

The sensillae in the sensory field on the ovipositor of *U. cardui* occur in pairs. Both pits and short setae occur. The pits form two concentric rings around the tip of the aculeus. Of the short setae (Fig. 6.13) one pair is located on each side of the aculeus. In the closely related species *U. affinis* four different types of sensillae were found on the ovipositor (Zacharuk *et al.*, 1986). The two types of pegs on the aculeus of *U. affinis* resemble the ones found on the ovipositor of *U. cardui,* although it was not possible to distinguish between the two different types of pegs here. In both species these short setae are arranged in the same way. Additionally pits also occur in both species. The pits of *U. affinis*, which Zacharuk *et al.* (1986) described as campaniform sensilla, were also arranged pair-wise concentrically to the tip of the aculeus. *U. affinis* does not have as many pits as *U. cardui* on the ovipositor. In the latter species eight pairs were counted, while *U. affinis* possessed only four pairs (Zacharuk *et al.*, 1986). In agreement with Zacharuk *et al.* (1986) these pits are henceforth referred to as campaniform sensillae and the short setae as uniporous pegs.

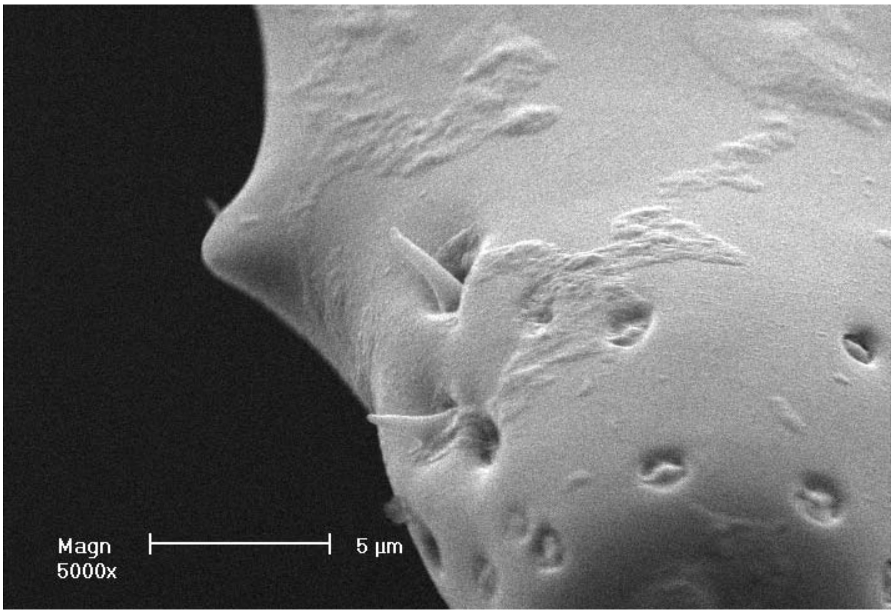

Fig. 6.14: Different sensillae types concentrated on the tip of the aculeus. Two morphologically different sensillae types can be distinguished: short setae and pits.

The campaniform sensillae as well as the uniporous pegs have a diameter of 1-2µm (Fig. 6.15, Fig. 6.16). The latter are about 1.5µm long and have a short seta ending in a blunt tip (Fig. 6.16). Setae similar to those in *U. cardui* and *U. affinis* have also been found by Brown & Anderson (1998) and van Lenteren (1972) respectively in *Trybliographa rapae* and *Pseudeucoila bochei* (Hymenoptera: Cynipidae). Campaniform sensillae of a similar shape as those in *U. cardui* and *U. affinis* but with a diameter of 5µm have been found in the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) (Salama & Abdel Aziz, 2001). In all these different insect species the authors described a relatively short peg, which was characterized by a deep socket (see also Casiraghi *et al.*, 2001; Hawke *et al.*, 1973; King & Fordy, 1970 and Rahmann *et al.*, 1998). This is a typical structure for mechanosensillae. Brown & Anderson (1998) and van Lenteren (1972) however, proposed that these pegs might have gustatory as well as thermosensitive functions. Temperature conditions inside the axillary buds of the creeping thistle may not be as important for *U. cardui* as the water content of the tissues (H. Zwölfer pers. comm., see also Hattori, 1988) and Horner & Abrahamson, 1992) due to the preference of *U. cardui* for thistles growing in relatively wet locations (Rotherey, 1986, compare chapter 2.1.2). It is thus tempting to speculate that the different sensillae on the aculeus of *U. cardui* do not only function as mechano- and chemosensores but as well as hygrosensors, as it has been shown for *Periplaneta americana* (Blattariae: Blattidae) (Altner *et al.*, 1977).

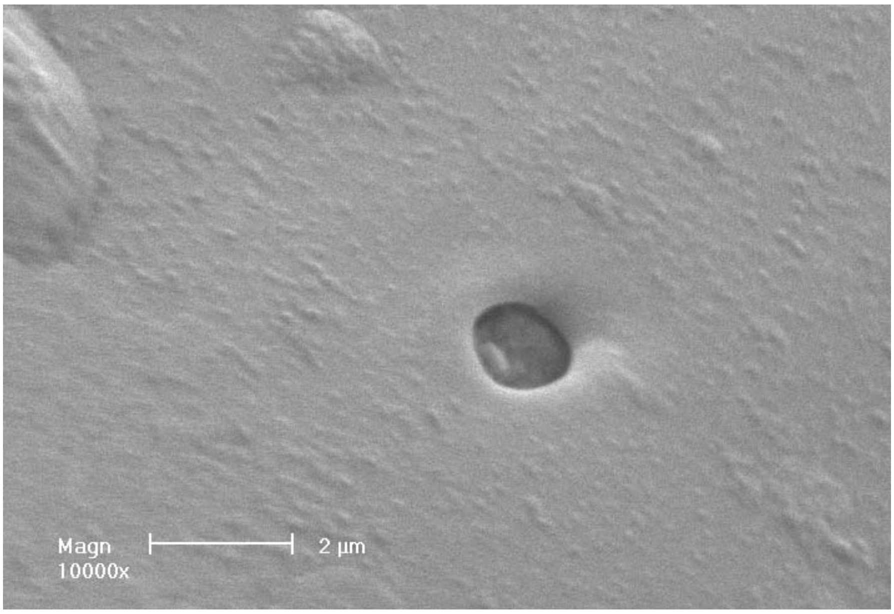

Fig. 6.15: Close-up of a campaniform sensilla on the tip of the aculeus of *U. cardui.*

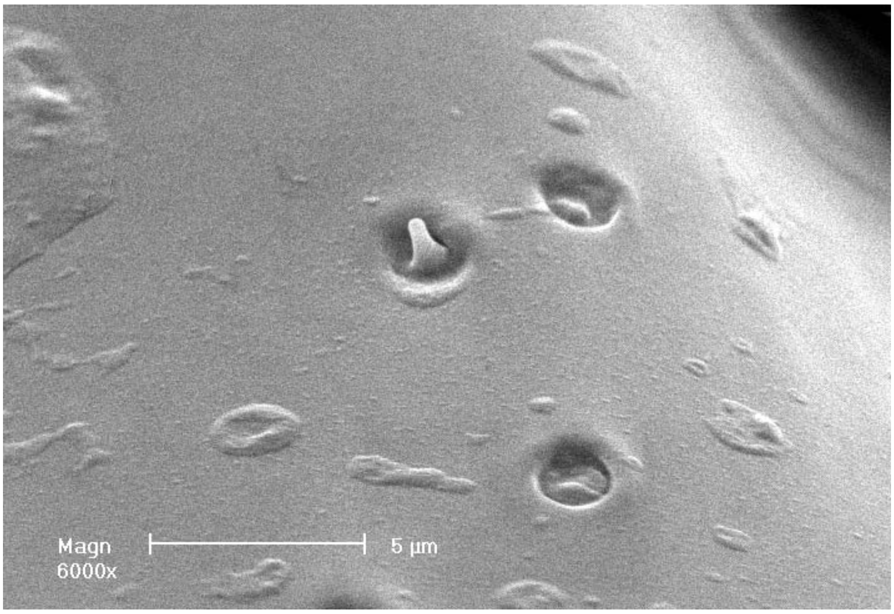

Fig. 6.16: One uniporous peg and two campaniform sensillae on the tip of the aculeus of the ovipositor of *U. cardui.*

6.4 Acknowledgements

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7 The selection of a suitable axillary bud for oviposition by *Urophora cardui* females (Diptera: Tephritidae)

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After the selection of a suitable host plant a galling insect has to analyse the quality of different plant organs to perform the decision for an oviposition site. The larvae are not able to leave the gall and move to better feeding sites, thus this last step in the foraging of a galling insect has a high influence on larval performance. Usage of the inner structure of the apical meristem of side shoots by the specialised gall fly *Urophora cardui* was analysed here.

The females of *U. cardui* are able to distinguish apical meristem of different sizes, which indicate different developmental stages. A significant difference was found between the diameter of the apical meristem of rejected and accepted axillary buds. Females of *U. cardui* discriminate because only apical meristem of a specific developmental stage permit successful gall-induction. Apical meristem with a diameter above 0.62mm appear to be of a suitable stage for larval development. No correlation between the diameter of the apical meristem of selected axillary buds and female weight at eclosure or head capsule width as indicators of female size could be found. It is possible that the females use the size of their ovipositor or tibia length during the selection process as a measurement. The clutch size is correlated with the diameter of accepted apical meristem: 48% of its variation can be explained by the size of the apical meristem. This further illustrates the high discriminatory ability of the *U. cardui* females in the last step of oviposition. Since this last part has a direct impact on larval performance, it is likely that the females use not only the cues provided by the size of the apical meristem of an axillary bud, but additionally its chemical composition or similar signals for its developmental stage.

7.1 Introduction

Mating and oviposition, which belong to the key behaviours exhibited by herbivorous or parasitic insects in ecosystems, can also be described as informational processes (Zwölfer, 1994): both behaviours are complex and often require a number of steps, which follow each other and set the initial conditions for the following elements of the behaviour (Hassell & Southwood, 1978; Mangel & Roitberg, 1989; Prokopy & Roitberg, 1989; Roitberg, 1985). During each step the individuals need to perceive information from their surroundings to estimate the quality of a mating partner or an oviposition site (Hassell & Southwood, 1978; Pyke, 1984). In systems that contain a limited number of species, like gall- or mine systems, the importance of these behaviours increases. Especially important in the case of a galling insect is a fine tuned discrimination of oviposition sites, since the larvae can never leave the gall, even if gall nutrition is not sufficient for their development (Ballabeni *et al.*, 2001; Zwölfer, 1994). Investigations of such systems are often performed to allow a better analysis of all interactions between different species (Fernandes *et al.*, 1999; Freese, 1995; Zwölfer, 1988). With respect to the influence on other relationships in the host-plant system, oviposition decisions are of particular interest, since they are interactions between the plant and the herbivorous insect, which represent the two lowest trophic levels (Achtziger, 1997; 1997; Hatcher, 1995; Price, 1992; Price *et al.*, 1980). The behaviour of a galling or mining insect and the plant chemistry both be very complex, involving not only behavioural plasticity on the insect's side, but also complex physiological plasticity in the plant and genotypic adaptations of both members of the interaction (Schoonhoven, 1990). Knowledge of all these traits would lead to a better understanding of insect–plant relationships as determinants of other interactions in an ecosystem.

An ovipositing female faces at least three consecutive decisions (Charnov & Skinner, 1985; Fletcher & Prokopy, 1991). First, the female has to select the particular habitat of the host (plant) species. Second, the female has to select one of the host (plant)s and third, the female has to select a suitable the oviposition site on a host (plant) and decide about the clutch size. The second step of the foraging for suitable host plants of the specialised gall fly *Urophora cardui* has been extensively covered in the last chapters (3, 4, 5). This chapter concentrates on the third step of the foraging for host plants, the selection of one particular oviposition site on a suitable host.

The third step of the selection process directly sets the initial conditions for larval development. Successful larval development increases the fitness of the females (Nylin & Gotthard, 1998; Sitch *et al.*, 1988; Smith & Fretwell, 1974): careful selection of the oviposition site is therefore crucial for the females (Mayhew, 1997; Sitch *et al.*, 1988; Weis *et al.*, 1988). They should be able to judge not only the quality of a certain host plant, but also of different plant parts, since this has a major impact on larval performance. Plant quality (or plant vigour) is defined as the supply of nutrients, water content and toughness of the tissues (Price, 1991; Stamp & Bowers, 1990 and references within). Often it is associated with nitrogen-content (McNeill & Southwood, 1978; Müller & Steinger, 1990; White, 1993). In the case of a certain plant organ other substances or structures probably serve as a cue for the females during the selection process.

It is generally assumed that females of herbivorous insects are able to make an optimal choice with respect to larval performance (Jaenike, 1990 and references within), as reported by Craig *et al.* (1989); Diaz-Fleischer *et al.* (1999); Lalonde & Roitberg (1992) and Via (1986). In the case of *U. cardui* this would signify that the females are able to estimate the developmental stage of the axillary bud. Older axillary buds, which have already developed a young shoot and several leaves were never probed by females (see chapter 3). Axillary buds, which are still very young do not provide the appropriate conditions for gall induction (Lalonde & Shorthouse, 1985). The specificity of monophaghous herbivorous insects to a certain developmental stage of the host plant is often very precise (Abrahamson & Weis, 1987; Strand, 1986), and this is so for *U. cardui* (Zwölfer, 1979). Since the information provided by phenological cues is quite reliable, Lalonde & Roitberg (1992) state, that a gallfly should be highly selective due to its low fecundity. Furthermore, low time costs, compared to the limitation by low fecundity, allow the females to spend a high amount of time on a high level of discrimination (Diaz-Fleischer & Aluja, 2003; Ives, 1989; Roitberg, 1989; Weis *et al.*, 1985). In *U. cardui* this is easily demonstrated by the long time females spend probing different axillary buds on a chosen host plant (see chapter 3).

Schmidt (1994) states that females of several parasitoid species often use measures of host volume as a component for host recognition and an aid to the determination of clutch size and sex ratio. *U. cardui* females may use similar cues, as galling insects and parasitoids share many general characteristics (Rosenheim, 1996). It is likely that the females use the information of several senses as during the selection of a suitable host plant (see chapter 5) due to the high dependence of larval performance on female choice.

In this chapter, the impact of the size of the apical meristem of axillary buds on female choice of suitable oviposition sites was studied. Additionally the influence of the size of the apical meristem of the axillary bud selected on clutch size was analysed, to compare the results with those obtained by Freese (1997) and Freese & Zwölfer (1996).

7.2 Material and Methods

The biology of the study system has been described in chapter 2.1.2.

7.2.1 Origin of plants and flies, general rearing

Thistles and rhizomes were collected in early spring, when the first leaves of the plants were visible. Different habitats were used for plant collection: a wet meadow, a large border of a crop field and the bank of a brook. Several preliminary experiments showed that the females did not discriminate between plants of different origin, thus the results of plants from different origins were pooled in all experiments.

Thistle growing and synchronization with emergence of the flies was performed with a similar breeding strategy as used by Sakuth (1996).

Plants were potted in soil (5 parts neutral humic soil, 2 parts pumice, 2 parts lava, 1 part loess, 2 parts peat and 2500g Osmokote^{TM} (a long-time fertilizer)/1m^3 soil) and kept in the open to provide semi-natural conditions. In autumn and winter plants were grown in the greenhouse at L:D 15 (23°C):9 (18°C). The light conditions were achieved by an array of mercury lamps (Osram Power Star HQ I–TS 250 WINAL with a minimal light intensity of 400µEm^{-2}s^{-1}). The plants were watered twice a day to avoid drought stress, which would have affected female preference and larval performance. The thistles were repotted to larger pots after approximately two weeks growing and again two to four weeks later. The final pot size was three or five litres, according to plant size. Each plant was numbered, the height measured, and axillary buds and flower buds were counted before usage. The axillary buds were counted from the oldest leaves up to the top of the plant and numbered in the same direction. For multi-stemmed plants only the height of the largest shoot was recorded, but total shoot number was noted and all buds were counted as well. No thistle was used twice.

The *U. cardui* flies originated from several small populations within approximately twenty kilometres around Bayreuth, Germany. Some of the flies were part of the newly established laboratory population, although this experimental population could not be maintained without new flies from the field. The galls were collected in late summer and autumn and stored in the refrigerator until initiation of eclosure.

The flies emerged from these galls in Petri-dishes (ø 14 cm) and were then marked individually with a two colour-code (Hobbyline, Acryl - Colours in light green (10), blue (24), red (15), yellow (03) and orange (04)) on the wings. No interference of the marking with the behaviour was observed in preliminary comparisons. The same colour codes were used again, if new individuals emerged from galls after the original possessor had died.

Between the experiments the flies were held in plastic cages (volume: 6l, Fauna box by Savic, Belgium) lined with regularly moistened cellulose tissue. They were fed *ad libitum* with

50% honey in water solution or sucrose-solution 67.5% (w/v) (Freese & Zwölfer, 1996; McCaffrey *et al.*, 1994). Water was also provided *ad libitum*. A maximum of 15 flies was kept per box. Two of the boxes were for single sexes, containing only males or females, to have a stock of virgin, naïve individuals. In most cases prior to experiments the flies were not confronted with thistles, in order to enhance individual motivation.

7.2.2 The selection of suitable axillary buds

Female selection behaviour on a host plant already selected was observed. The size of rejected and accepted apical meristems was compared to test its influence on the selection process.

To provide a semi-natural environment a small observation cage (25cm x 35cm x 100cm) was installed outside in a natural stand of thistles. Inside the cage, all naturally growing plants were removed. Preliminary experiments indicated, that the presence of more plants than the two presented enhanced the motivation of the tested individuals.

Two potted thistles of identical height and developmental stage were put in the cage, together with a small vial filled with sugar-water (67.5% w/w). Females and males of approximately the same age were released into one cage. The sex-ratio was 1:1, as in nature (Schlumprecht, 1990; Zwölfer & Schlumprecht, 1993). Between four and eight individuals were placed in the observation cage. In preliminary experiments it has been shown, that observation of more than eight individuals (= four females) is not practical. Observations lasted from 09:00-15:00 (CEST), when the flies have the highest activity on sunny days (H. Zwölfer pers. comm., personal observations). All behavioural acts of two selected females were recorded and timed to a resolution of one second. The remaining one or two females were also observed, but without recording every single behavioural act (see chapter 3.2). Each probe and each oviposition (see chapter 3.1.1) of any of the two to four females was recorded separately by the thistle individual, the colour code of the female and the identity of the respective axillary buds. Eight observation sessions where performed using 16 thistles in total.

At the end of the six hours observation time the flies were captured with a poster (Hölldobler & Wilson, 1995). All probed axillary buds and all axillary buds accepted for oviposition were marked, respectively, with a white or black marker (edding 3000) to allow easy differentiation between the visited and unvisited buds on the plant.

All axillary buds marked were dissected with a razorblade and at least one longitudinal section of each axillary bud was fixed on a microscope slide. These sections were examined with a binocular microscope (Wild M38, Heerbrugg, Switzerland) at 16-fold magnification.

The diameter of the apical meristem was measured with a calibrated ocular micrometer (Wild 10/21B). The eggs in the apical meristem accepted for oviposition were counted in order to analyse the possible influence of size of the apical meristem on *U. cardui* clutch size.

Data were organized and pre-examined in an Access–Database (Access 2000, Microsoft corporation) and afterwards transferred into SPSS (10.0 SPSS Inc.). Prior to a t-test (to ensure its applicability), a Levene test of variances (Dytham, 1999; Sokal & Rohlf, 1995) was carried out (F=2,604; p=0,115; n=38). Additionally a logistic regression analysis was performed in Statistica (Statistica 5.5 Statsoft) to calculate a possible threshold value (Sokal & Rohlf, 1995) of the diameter of the apical meristem, which may influence the behavioural decision of the individual females. The resulting formula of the logistic regression analysis is $y=\exp(b_0+(b_1)*x)/(1+\exp(b_0+(b_1)*x)$ with the threshold value $x_{thres}=-b_0/b_1$.

In order to analyse whether the females are able to use their own body size as scale for the examination of the diameter of the apical meristem a Spearman–Rank–Correlation was performed in SPSS (Dytham, 1999). Body weight of the females and their head capsule width were both sampled as an overall size indicator (see chapter 2.3.4). Possible correlation of size of the apical meristem and clutch size were also analysed via Spearman-Rank-Correlation.

7.3 Results

On twelve plants the females probed and/or laid eggs, while the remaining four were unused, although some of the males established territories there. The data presented here were obtained from 24 females of different age classes (between 4 and 14 days old). All these females had previous experience in egg deposition and were mated at least once.

19 accepted and 19 rejected apical meristem of different thistles were dissected. The mean of the rejected apical meristem was 0.47mm (±0.246). The mean of the accepted apical meristem was 0.81mm (±0.222). Comparison of the rejected and the accepted axillary buds revealed a highly significant difference in respect to the diameter of the apical meristem (t-test: T=-5.704; df=1, n=38, p<0.001, Fig. 7.1).

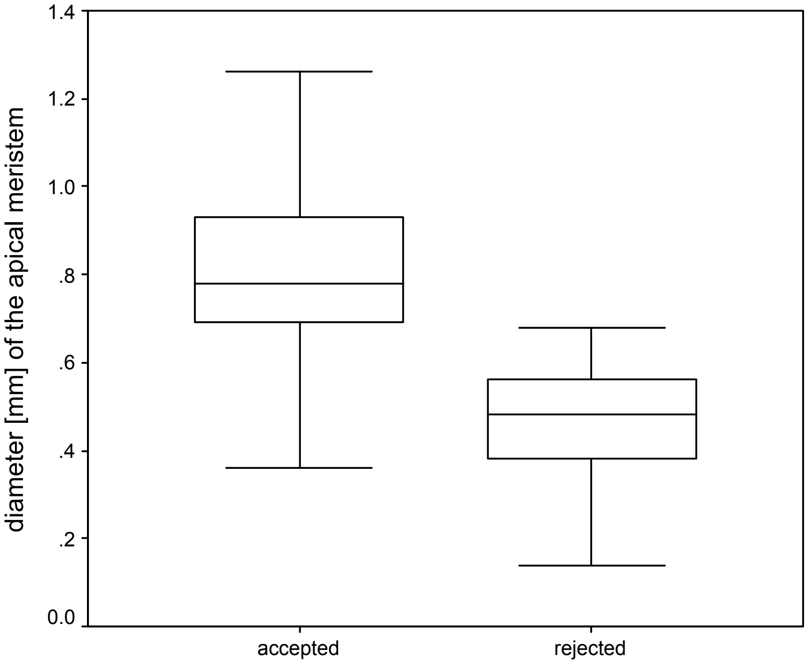

Fig. 7.1: Comparison of the diameters [mm] of rejected (n=19) and accepted apical meristem (n=19). Lines in the boxes=median; boxes=25%-75%; whiskers=minimum and maximum diameters.

Alongside the presence of this significant difference in diameter of rejected and accepted apical meristem, it was possible to analyse the signal that individual females may use during their decision process by a logistic regression analysis, revealing a threshold value of 0.62mm diameter (Fig. 7.2). Apical meristem of this particular size have 50% probability of being selected as oviposition site by an *U. cardui* female. Above this threshold the probability of an apical meristem being accepted as oviposition site increases, below that it decreases.

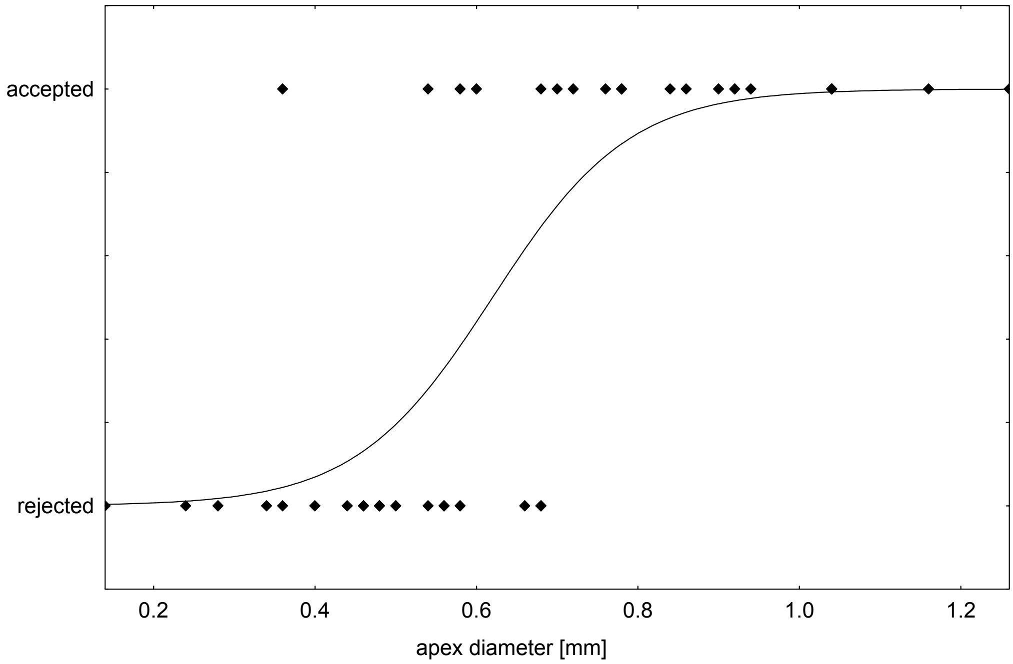

Fig. 7.2: Logistic regression analysis of rejected (n=19) and accepted apical meristem (n=19). Some of the values occurred several times and are thus represented by a single square. y=exp (-7.32122+(11.80776)*x)/(1+exp (-7.32122+(11.80776)*x)

The females of *U. cardui* are not able to measure the diameter of the apical meristem absolutely, therefore it is probable that they use their own body size as a reference, although no correlation was obtained between weight at eclosure and the size of the apical meristem (Fig. 7.3), (R_s=-0.008, df=1, n=24, p=0.966) chosen by the respective female. The results of a correlation between diameter of the apical meristem and head–capsule width as a scale of female size were similar (R_s=-0.122, df=1, n=24, p=0.61).

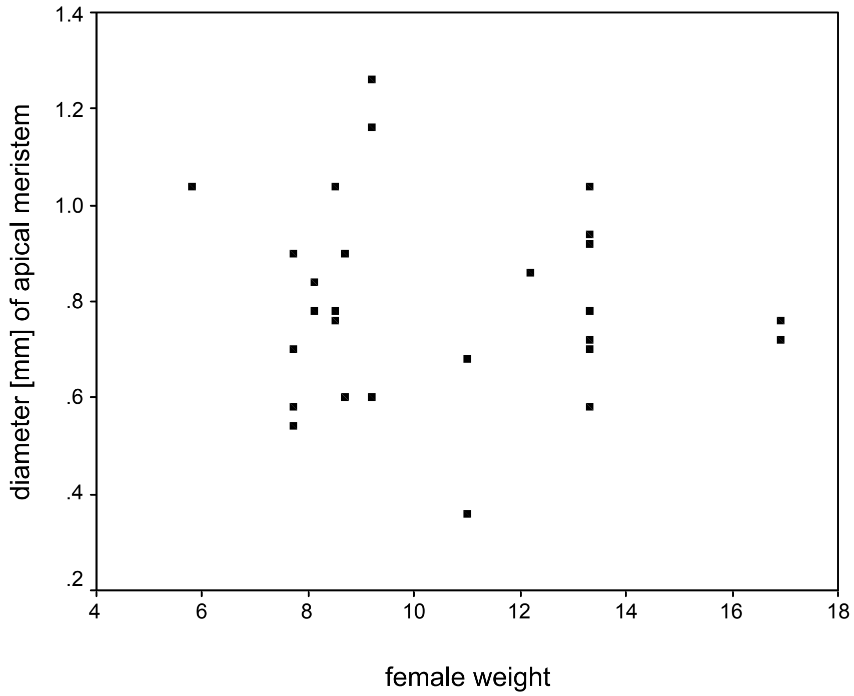

Fig. 7.3: Scatter plot of the relationship between female weight [mg] at eclosure (as an indicator of female size) and the diameter of the accepted apical meristem [mm] (n=27, three females oviposited in two apical meristem of different size and several females oviposited in five different apical meristems).

Concerning the relationship between the diameter of the apical meristem and clutch size, a significant correlation was obtained in both tests: if the rejected apical meristems are included as a clutch size of zero, 75% of the variation in the clutch size can be explained by the diameter of the apical meristem (R_S=0.745, n=38, p<0.001) (Fig. 7.4). If only the accepted apical meristems were analysed to exclude the yes/no choice of the females, 48% of the variation in clutch size can still be explained (R_S=0.48, n=21, p=0.028).

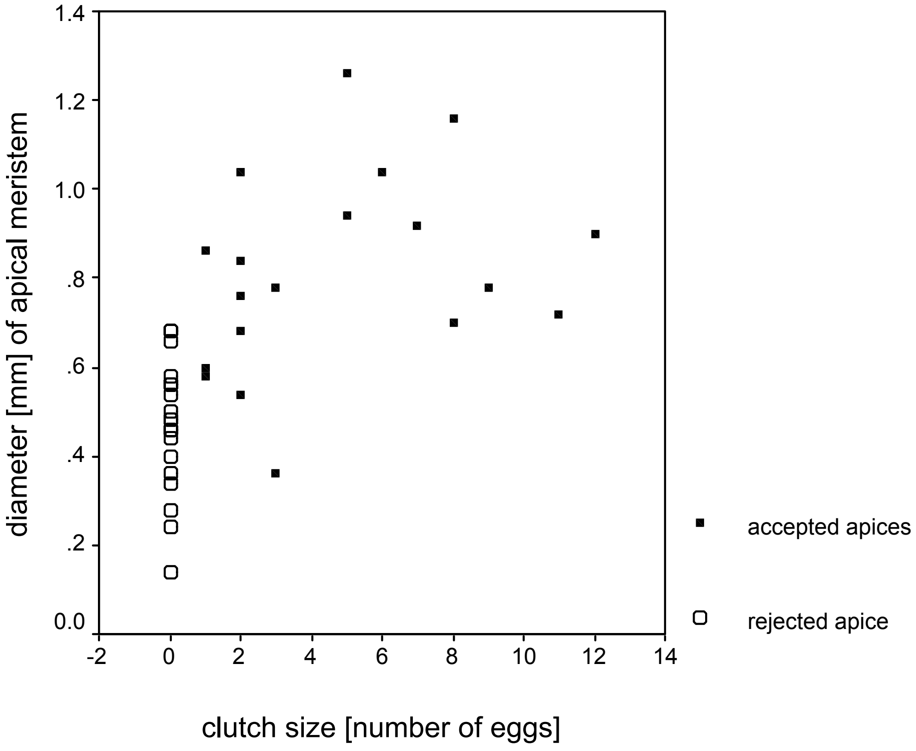

Fig. 7.4: Correlation of the number of eggs and the diameter of the apical meristem. Diameters of rejected apical meristem (clutch size=0, n=19)=○; diameters of accepted apical meristem=■ (n=19).

7.4 Discussion

The diameters of accepted and rejected apical meristem were significantly different, while those above a threshold of 0.62mm have a higher probability of selection as an oviposition site over those with a diameter below the threshold. The respective clutch size is adjusted to the size of the apical meristem. It is likely that females use their own body size as a reference for measurement of the apical meristem (see Jones, 2000 and references within), but obviously both the body weight at eclosure and the head capsule width are bad scales of female body size in this context: probably the females use their tibia length or ovipositor length instead. A different hypothesis explaining the lack of a significant correlation between the scales of female size used here and the size of the apical meristem they selected for, may be that the diameter of the apical meristem is only an indirect cue used by the females. Apical meristem of different size represent axillary buds of distinct developmental stages: there may be other indicators of the developmental stage, for example the chemical composition of the tissue.

A problem encountered repeatedly is the influence of the motivation of the females on the obtained results. Motivational states always differ individually. It has been stressed for a variety of insect species, that the behaviour in response to a signal strongly depends on the motivation of the individual (see for example Hölldobler, 1999). The wide range of the diameter of accepted apical meristem in comparison to the range of rejected apical meristem may indicate the influence of motivation on female choice. Here motivation would mainly constitute egg-load and the time passed since the last oviposition of the individual. A female with a high egg-load would accept oviposition sites of less suitability (see for example Rojas, 1999). Mangel & Roitberg (1989) expect host acceptance to depend on physiological and informational (knowledge of suitable host plants) stages. They developed a model, which shows how the plasticity in oviposition site selection arises from fitness maximizing behaviour; changes in internal physiological stage may modify preference by altering the perception of external cues or reducing thresholds for particular responses. The overlapping zone in the logistic regression (Fig. 7.2) illustrates that this may also be the case in *U. cardui*. So far the linkage of physiological and behavioural changes is unknown (Fitt, 1986 and references within).

For some other dipteran species that parasitize flower heads it has been shown that the length of the bracts and the achenes can be used as a cue in host plant recognition (Burkhardt & Zwölfer, 2002; Straw, 1989). It was thought, that the females are able to use their own size as a reference for the measurement of host plant structures. Unfortunately definite experiments on *how* females of dipteran species may be able to measure structures of the larval host (plant) are still lacking, although there exist several studies, which stress the influence of structural cues. In *Tephritis conura* (Diptera: Tephritidae) for example it was described that the females only accept a very narrow spectrum of developed buds (Romstöck & Arnold, 1987). Since the females lay their eggs in the ray florets of buds of *Cirsium heterophyllum* (Asteraceae), which have a certain length, the length of their ovipositor is a limiting factor. A similar situation occurs in parasitoids of the genus *Eurytoma* (Hymenoptera: Eurytomidae), they are limited by the gall size of their hosts due to their ovipositor length (Hoffmeister & Gienapp, 1999; Hoffmeister *et al.*, 1999; Weis *et al.*, 1985). It is unlikely, that ovipositor length is limiting for *U. cardui*, since the females reject buds that are of a smaller size. But like *Tephritis conura* and *Eurytoma* spec. the females determine the size of the host (plant organ) they oviposit into. Several studies on other insect species report comparable host cues that females are able to use for their choice of oviposition sites. In *Hypoderma lineatum* and possibly other *Hypoderma* species (Diptera: Oestridae), the females measure the diameter of surface hairs of cattle they lay their eggs onto (Cogley *et al.*, 1981; Jones, 2000). Another case in which host size is important during selection for oviposition sites was analysed by Askew & Shaw (1979) in parasitoids of *Phyllonorycter* sp. (Lepidoptera: Gracillariidae) that are leaf miners on oak and birch. The ectoparasites especially have to discriminate between small hosts, where they deposit only male eggs, and larger hosts, where they are able to deposit female eggs (Askew & Shaw, 1986). Thus usage of tactile cues provided by the host (plant) structure during selection for oviposition sites is common in various insect species. It is therefore likely that females of *U. cardui* are also able to measure the size of the apical meristem and use it as one indicator of the developmental stage.

Interestingly the correlation between clutch size and size of the apical meristem, which was obtained here revealed a tighter relationship than that proposed by (Freese, 1997; Freese & Zwölfer, 1996). They found only 21%, while in this study 48% of the variance could be explained. Since the measurement was performed in a similar way, it is likely that conditions of the thistles used in their experiments and here were in some way different (see also the discussion about the influence of a very high sample size in (Wiley, 2003).

Host plant recognition becomes more subtle from step to step (Zwölfer, 1968). This implies, that the females have to use more and more finely tuned estimations of host (plant)-

suitability. This task would be much easier to perform, if the females use multisensory input (for example Aluja & Prokopy, 1993; Beehler *et al.*, 1993; Fenemore, 1980; Ngi-Song *et al.*, 1996; Strom *et al.*, 2001). Therefore it is expected, that the females might use not only the size of the apical meristem as a reliable cue, but also chemical composition of the interior of the axillary bud. This would also be in accordance with the receptors that were identified on the ovipositor of the *U. cardui* females (see chapter 6).

As has been shown in this and in previous chapters, even within the general category of a plant part (for example leaves, or axillary young side shoots), females can be highly selective in their choice of oviposition locations. Short-term studies of only a few years are probably insufficient for evaluation of the selection pressures and constraints that influence this high selectivity for oviposition sites within plants (Thompson & Pellmyr, 1991). This implies that the present investigation may have only scraped at the surface of oviposition strategies. Additionally it is difficult to extrapolate from the behaviour of the flies in observation cages to the behaviour in nature (Prokopy & Roitberg, 1989; Roitberg, 1988). It would thus be useful to confirm the results in a long-term field study, although this may be difficult to perform with respect to manpower and funding.

It would be interesting to determine, if larval performance is correlated with female choice, as has been shown in other insect species (Rausher & Papaj, 1983; Thompson, 1988). This would give valuable hints about how the females adjust their choice to the needs of the larvae. But in galling or mining insect species it is not yet possible to transfer the larvae from one plant to an alternative host. Thus the relevance of the developmental stage of the host plant can only be analysed indirectly. The synchronisation of herbivorous insects and their host plants and its importance for larval performance and thus for population structure have been stressed for several species related to *U. cardui* (Harris, 1980).

As in many other herbivorous and parasitoid insect species, the females of *U. cardui* are able to use tactile and structural cues of their host plant to select suitable oviposition sites. In the last step of their complex foraging strategy they are able to use the signals provided by one single host plant organ. At this point they set the initial conditions for gall induction and thus larval development (Lalonde & Shorthouse, 1985). The importance of this last assessment of host plant quality is also highlighted by the extensive probing behaviour, which can last several hours (see chapter 3.1.1). A high ability to analyse the quality of the axillary bud and to estimate its future potential is necessary for the females to enhance larval performance and thus their own fitness. Therefore the diameter of the apical meristem and possibly also its chemical composition provide reliable cues for the female.

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8 Résumé: Model of the foraging for oviposition sites by *Urophora cardui*

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Host plant recognition and selection by herbivorous insects is divided into several different steps that can be examined individually. Each of the chapters of this thesis analyses one of the different steps of the host plant selection of the specialised gallfly *Urophora cardui,* for a suitable oviposition place on the larval host plant *Cirsium arvense*. The different tasks an *U. cardui* female has to perform in relation to male choice of a host plant as a territory (see **chapter 5**) will now be recapitulated. Based on these findings a possible model of the host plant selection of a specialised herbivorous insect will be presented.

According to the type of study system, host plant selection by a herbivorous insect and the comparable host selection of parasitoids (Rosenheim, 1996) are divided into a different number of distinguishable steps. In most of the analyses covering host (plant) selection at least three different tasks are mentioned: 1) selection of a certain habitat; 2) selection of one particular host (plant); and 3) selection of a suitable host (plant) organ, which is tightly linked to decision about the clutch size (Fletcher & Prokopy, 1991; Nagelkerke, 1994; Roitberg, 1990; Rosenheim & Mangel, 1994). In the case of *U. cardui*, which was analysed here, additional steps need to be inserted into the foraging process, since the selection by males of territories on the larval host plants is connected to the oviposition-site selection of the females. It is thus necessary to include male choice divided into individual steps, into the ordered sequence of the foraging strategy as well.

As far as I know there are no other studies on specialised herbivorous insects, which use the host plant also as a rendezvous-place (Zwölfer, 1974), analysing the possible impact of male choice of territories on the selection of oviposition sites by females. At least in closely related species it can be assumed that there may be similar effects as shown here for *U. cardui*. It is possible, that male selection might not be as important in all of these species as it is in *U. cardui*: in *U. cardui,* host plant choice is not only important for search of mating partners, but thistles occupied and marked by males provide also information about safe oviposition sites on the host plant (see **chapter 2.1.2.2**), particularly in regard to spiders, which are important predators on *C. arvense* (Rauh, 1994).

In the following sections the different tasks of the foraging strategy of *U. cardui*, which have to be performed by males and females to enhance their fitness, will be presented in sequence. If the respective individual fails in one step, the subsequent ones have a high probability to fail also, diminishing the fitness of the particular fly. However the linkage of male choice for resource-based territories and female choice of larval host plants is presumably a flexible step.

8.1 Selection for a suitable host plant stand by the males

After their eclosion in spring, males of *U. cardui* need to establish a territory on a plant, which would be suitable for high larval performance, in order to get access to mating partners. The males already eclose inside a host plant stand; nevertheless there may be no host plants around that are at a suitable developmental stage. The young male will then disperse; it prefers host plant stands, which have a high water supply and where shading of the respective host plants is provided for example by trees (Zwölfer, 1982; Zwölfer, 1988). Additionally, according to its host plant template (Krebs & Davies, 1993; Tinbergen, 1934), it is sensitive to the volatile profile of its host plant *C. arvense*, and also to visual cues from the plant (see **chapter 5**). With the visual and olfactorial information provided by the host plants, the male is able to distinguish suitable plants from those, which may be already too old, or are too young. If the male encounters a host plant stand, which provides the correct characteristics, it will proceed to the next step in the process.

The foraging performed by males for a suitable host plant stand is schematically shown in Fig. 8.1.

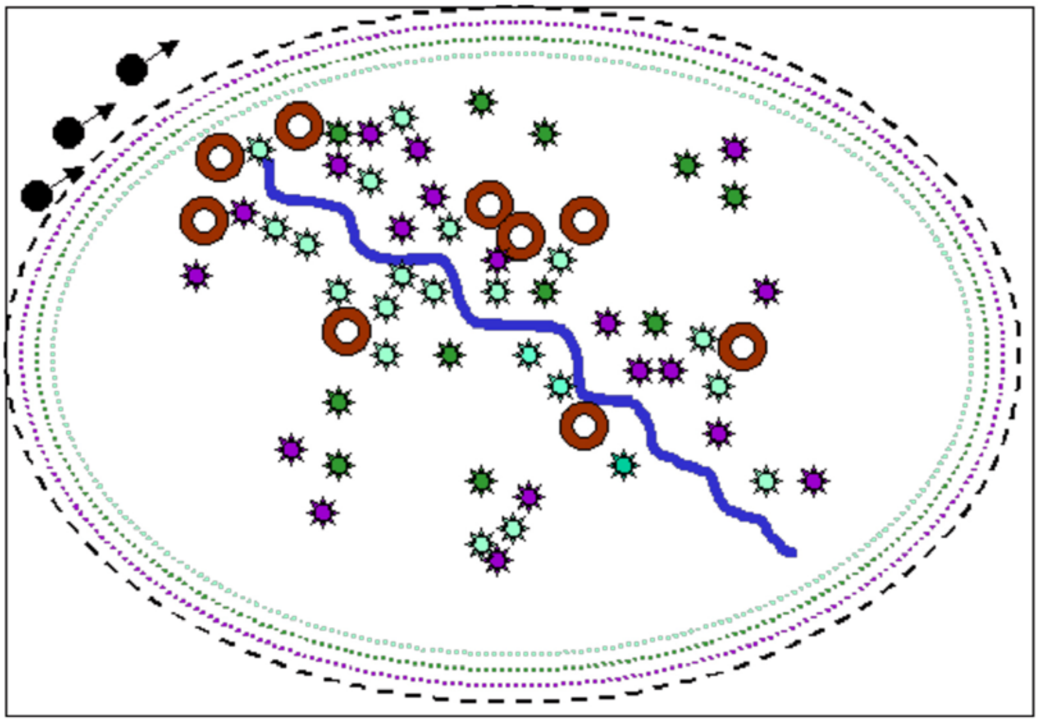

Fig. 8.1: Schematic drawing of the search for a suitable host plant stand performed by *U. cardui* males. Water supply (blue line), shading by trees (brown circles) and the general presence of the host plant constitute the habitat profile (black line). The different developmental stages of the thistles (which have been reduced to three to simplify the scheme) are symbolised by three different colours: light green, dark green and violet. The visual cues provided by these at least are different; the chemical information is presumably also altered at different developmental stages. There will be a difference in olfactorial cues especially between plants with and without flower buds. The different cues of the three developmental stages of the thistles are represented by the three dotted lines in the respective colours.

8.2 Selection by the males of a certain host plant as a territory

Inside a suitable host plant, stand the young male chooses one of the plants that would be suitable for oviposition. This decision is performed relatively quickly. The decision-time depends on the number of host plants, the number of non-host plants and the number of *suitable* host plants (see **chapter 4**). If some of the suitable host plants have already been selected and marked as a territory by conspecific males, the newly arrived male will prefer these as shown in **chapter 5**, which implies a high probability to alight on a spider-free substrate. If the plant is already occupied, the newly arrived male will first have to fight against the resident male (see **chapter 3**). Only after winning the contest it can mark the plant as its own territory. If it loses the fight it has to choose an other host plant instead, or to establish its territory on the lower part of the host plant if the size of the thistle permits more than one territory. The marking substance, 4-methyl-3*Z*, 5-hexadienoic acid, is present in a rectal gland (Frenzel *et al.*, 1990). The male applies it to the plant surface with the tip of its abdomen. The marking has to be renewed every couple of hours. There are no fixed marking patterns (see **chapter 3**), each individual male has its own characteristic marking mode.

8.3 Selection of a suitable host plant stand by the females

A newly eclosed female has to search not only for a suitable host plant stand, where it can deposit eggs, but also has to find a mating partner, too. Since the probability is high that mating partners will be present in suitable stands of host plants, the female starts searching for such a stand. According to its host plant preferences, it searches for the characteristics of an acceptable host plant stand, which include high water supply and shading (for example by trees) (Zwölfer, 1982; Zwölfer, 1988) as the two most important aspects not directly consequence on the host plant itself. Additionally, the female is able to orient on visual cues provided by the host plant (see **chapter 4** and **chapter 5**) and it is highly sensitive to the olfactorial profile of the male marking substance (see **chapter 5**). These parameters of female search for a suitable host plant are summarized as a schematic drawing in Fig. 8.2.

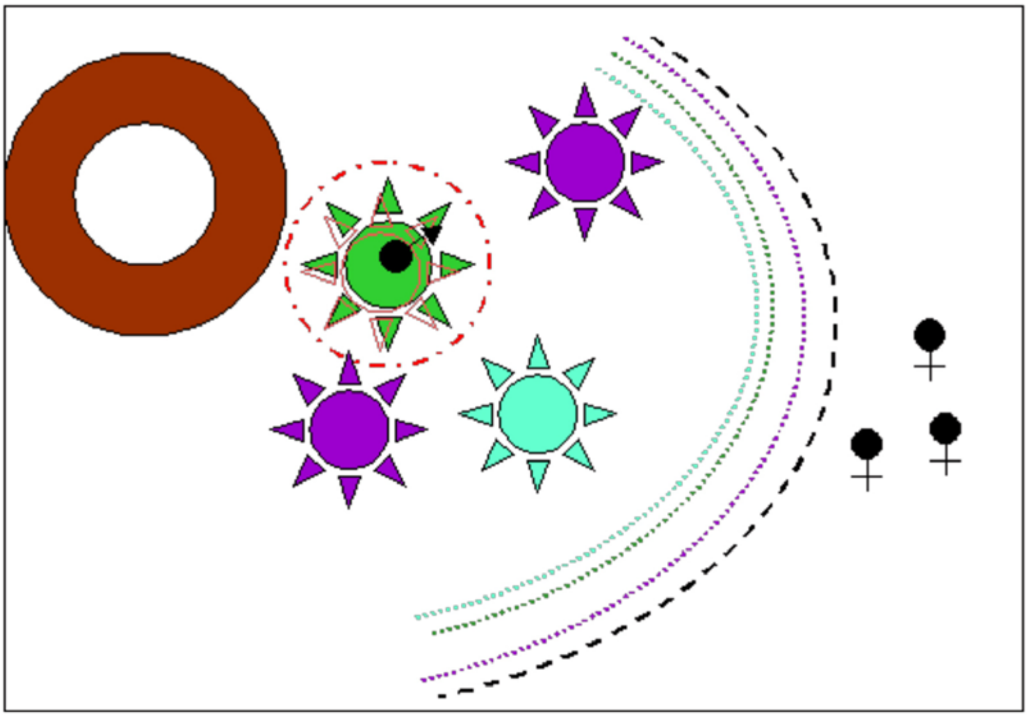

Fig. 8.2: Schematic drawing of selection for a suitable host plant stand by females of *U. cardui*. Water supply (not shown here), shading by trees (brown circles) and the general presence of the host plant constitute the habitat profile (black line). The different developmental stages of the thistles (which have been reduced to three to simplify the scheme) are symbolised by three different colours: light green, dark green and violet. The visual cues provided by these at least are different; the chemical information is presumably also altered at different developmental stages. There will be a difference in olfactorial cues especially between plants with and without flower buds. The different cues of the three developmental stages of the thistles are represented by the three dotted lines in the respective colours. The host plant, which has been marked by a conspecific male is highlighted by a red circle; thus, the olfactorial profile provided by the presence of the male marking substance is shown by the red line.

8.4 Selection by the females of a suitable host plant

Like the male, the newly arrived female will quite rapidly select one of the host plants (see **chapter 4**). The decision-time depends on the number of suitable (male-marked) host plants, the number of non-host plants and the number of unsuitable hosts. For its decision, the female uses a rigid host plant template (see **chapter 3**) This search image consists of the visual information from suitable host plants and the olfactorial profile from conspecific males, which have marked their territories. The female alights on the plant selected and starts to move around it searching for a suitable oviposition site (see **chapter 4**).

8.5 Selection of a suitable axillary bud and egg-deposition

The movement of the female around the host plant will be interrupted by probing bouts into axillary buds (see **chapter 3**), if the female has already mated. Otherwise, it will first mate with the resident male. With the receptors present on its ovipositor (see **chapter 6**), the female is able to measure the size of the apical meristem and probably also analyse the chemical composition of the adjacent tissue (see **chapter 7**). These measurements enable the female to assess the developmental stage of the respective axillary bud, which is important for the successful development of the gall and thus influences larval performance and the fitness of the female. If the axillary bud is determined to be suitable, the female will adjust the clutch size to the developmental stage of the axillary bud (see Freese & Zwölfer, 1996 and **chapter 7**), (Fig. 8.3), after having finished an extensive evaluation (see **chapter 3**).

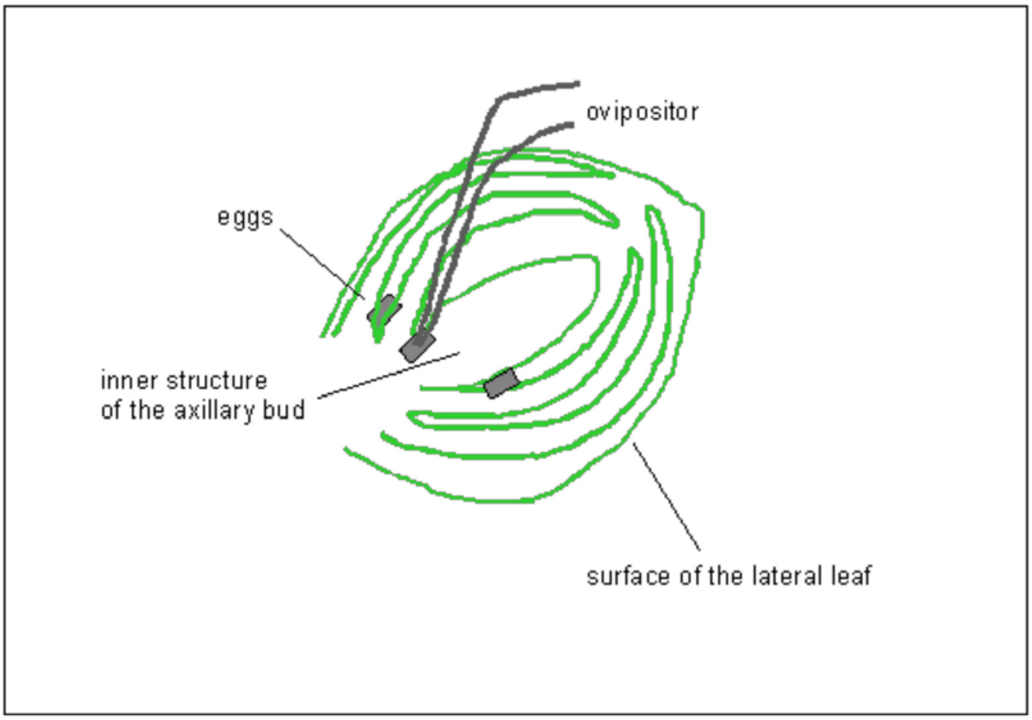

Fig. 8.3: The female probes the interior structure of an axillary bud, thereby using the information of the diameter of the apical meristem and chemical composition of the plant tissue. Afterwards the clutch size is adjusted to the quality of the bud, the latter determines its ability to develop into a gall, in which a high larval performance is guaranteed.

8.6 Summary

The foraging process performed by the specialised gall fly *U. cardui* for larval host plants can be divided into five consecutive steps, which follow each other in the described sequence: 1) selection of a host plant stand by the male, 2) selection of a *C. arvense* plant as a territory, and marking of that plant with 4-methyl-3*Z*, 5-hexadienoic acid by the male, 3) selection of a host plant stand by the female, 4) selection of a particular host plant by the female and 5) selection of a suitable axillary bud. After oviposition the female will usually mate with the resident male and then proceed to the next host plant or even to the next host plant stand. The whole model of the foraging process performed by males and females of *U. cardui* for *C. arvense* is summarized as a flow diagram in Fig. 8.4.

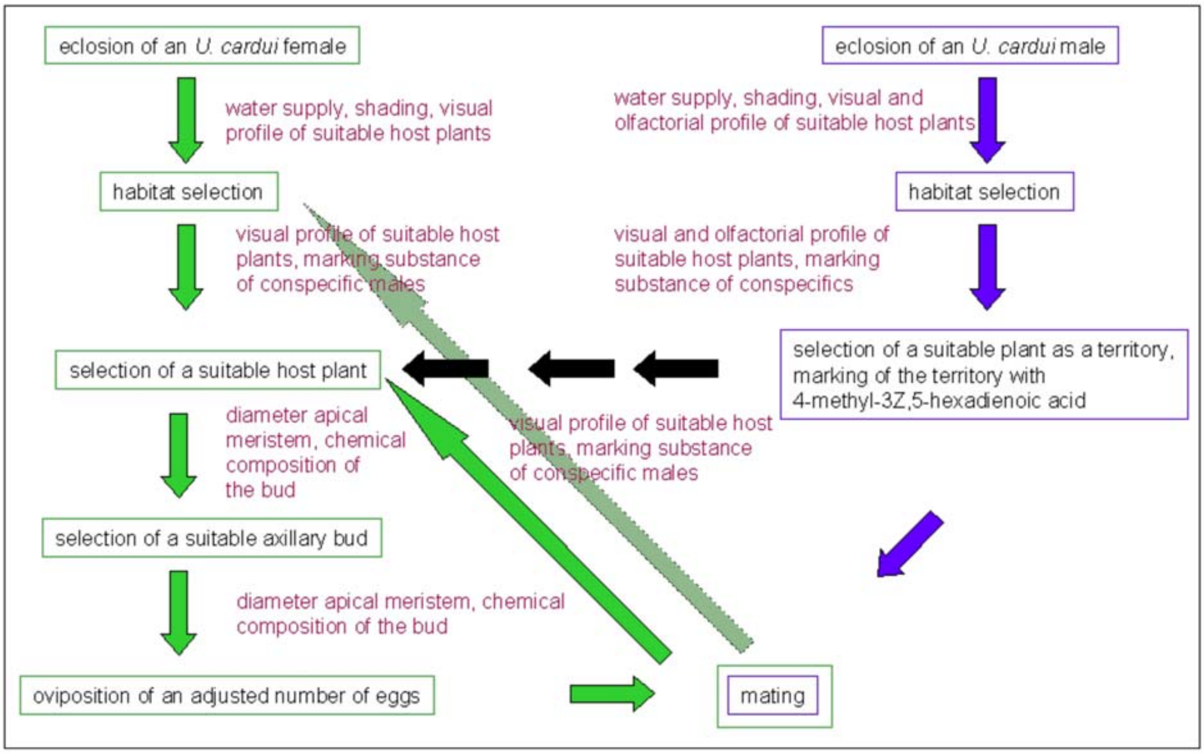

Fig. 8.4: Model of the host plant selection by *U. cardui* males and females. Blue arrows and boxes represent actions performed by the males. Green arrows and boxes represent actions, that are performed by the females. The light green arrow stands for a possible action. Black arrows show the effect one action has on another action. Text in dark red describes the cues, which are used by the males and females during a particular step of the foraging process.

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9 Synopsis

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Relations between insects and plants comprise the majority of interactions in all terrestrial ecosystems (Dettner *et al,* 1997; Schoonhoven, 1990; Schoonhoven & Jermy, 1998). Plants as the first trophic level constitute the base of all other trophic levels (Price *et al.*, 1980), thus all terrestrial animals interact either directly or indirectly with them (Bach, 1991; Gingras *et al.*, 2002; Strong *et al,* 1984). Both groups, the plant kingdom and the class of insects represent two very extensive taxa in respect to species number, species abundance and biomass. According to Schoonhoven & Jermy (1998) the biomass of all insects in the Brazilian Amazon outweighs that of total land vertebrate population by about nine to one. Similar or even more pronounced numbers apply to all terrestrial ecosystems (Schoonhoven & Jermy, 1998).

Plants serve as the primary, energy-rich resource for all heterotrophic organisms (Price, 1992). Herbivorous animals, especially insects have an influence on plants and are therefore one of the factors that have led to a great diversity of plants (Schoonhoven & Jermy, 1998). Insects depend on plants not only as the direct or indirect (in the case of predators and parasitoids) food source (Power, 1992), but also as a place for egg deposition (Fletcher & Prokopy, 1991; Hattori, 1988; Jaenike, 1978) and quite often also as a courtship and mating place for the adult insects (Zwölfer, 1974). The interrelationships between plants, herbivores and their enemies encompass several areas of theoretical ecology (see also **chapter 1**) that are seldom viewed together. Examples are the theory of plant chemical defence (Feeny, 1990), dynamic population models (Pimm, 1982), food web theory (Price *et al.*, 1995), succession (Tscharntke, 1990), habitat stability (Hurd *et al.*, 1971), behavioural ecology (Price *et al.*, 1980), sensory physiology (Harris & Miller, 1988), reproductive physiology (Strand, 1986), biochemistry (Carlsson & Hansson, 2003) and applied entomology (Robert, 1986). The study of foraging strategies is positioned in the center of this multifaceted network of theoretical aspects. It makes studies in this area most fascinating, but also very complex. It is difficult to incorporate all the mentioned adjacent theories and control for influence of all these topics. Thus, many aspects of the insect-plant interactions still remain unknown, but since the interactions of insects with plants are a key element in many terrestrial ecosystems there is a need to explore, analyse and understand those interactions. Nevertheless studies that are performed in a large ecosystem with many unknown processes are difficult and often unsuccessful (Hall & Raffaelli, 1997): scientists always search for suitable models, which provide the important aspects in question, but contain only a small number of involved species and are subject to less influence from the world outside (Angermann, 1984; Hurd *et al.*, 1971; Jeffries & Lawton, 1984). One potential model system is plant galls. In the previous chapters the plant gall system of the specialised gall fly *Urophora cardui* and its host plant *Cirsium arvense*, an aggressive weed species, was investigated as an example of the foraging process for larval host plants performed by a specialised herbivorous insect.

Resource-rich environments like agro-ecosystems or old-field habitats favour rapidly growing species, like *C. arvense*. In these environments, *C. arvense* can reach tremendous densities.

However, due to its gall characteristics (see **chapter 2.1.2**) *U. cardui* is not able to maintain permanent populations on plants growing in agro-ecosystems, since due to anthropogenous disturbance in most of these habitats there is no continuous supply of ramets; additionally water supply is low and shading by trees rare. *U. cardui* is thus mostly restricted to relatively undisturbed thistle stands where *C. arvense* is not usually the dominant plant and occurs in lower density in the respective patch (Zwölfer, 1982; Zwölfer, 1988; Zwölfer & Arnold-Rinehart, 1993). This requires specialised host plant finding capabilities (see **chapter 1**). For herbivorous insects it is easy to locate plants, which are growing in dense groups (Price *et al.*, 1980; Price *et al.*, 1990), since the probability that the neighbouring plant is of the same species is high. The detection of the plant in question is easy, as population densities of the other species are low (Jones, 2000). The opposite applies to herbivorous species colonising plant species that grow among many other species and are not abundant (Jones, 2000) As shown here, the host plant recognition ability of *U. cardui* is well adapted to the latter conditions (see **chapter 4, 5 and 7**). The males and females are not only able to detect their host among other plants (see **chapter 5.3.2**), they pre-select a certain plant before they land on it (see **chapter 4.3.1**). The results presented here suggest that they are already able to perceive the suitability of possible host plants from a distance of 0.8m (**chapter 5**), presumably already from 1-2m (see **chapter 4**). This study is the first to emphasize the choice of males (see **chapter 5.3.5**). Since the host plant *C. arvense* serves also as a mating place for *U. cardui,* males also need to select a plant that is suitable for oviposition, to attract females. Therefore the males use their olfactory receptors and vision to choose one plant as a courtship and rendezvous territory (see **chapter 5.3.3 and 5.3.4**). The males preferred plants that had already been marked by a conspecific male. This result indicates that male competition is preferable to the risk of predation by spiders (see **chapter 2.1.2 and 5.3.5**).

The females prefer plants, which were already marked by a conspecific male. It is assumed that search for a mating partner is not the only reason, but that avoidance of spiders, which occupy thistles and prey on *U. cardui* (see **chapter 2.1.2**) plays an additional role for this behaviour (M. Rauh unpublished data). It was shown here that the olfactory system of the females is extremely sensitive to perceive plants that have been marked by a male in advance (see **chapter 5.2.3.4**).

The high demands of *U. cardui* on the quality of its host plant (see **chapters 3, 4, 5 and 7**) leads to a low impact on *C. arvense* populations in agricultural fields (Goeden, 1987; Peschken & Derby, 1997; Peschken *et al.*, 1982). It is possible that these aspects of the foraging strategy of *U. cardui* have at least delayed its effect on the host plant in Canada. Recently there are hints, that the negative impact of *U. cardui* on *C. arvense* is now increasing (Harris, P. and Zwölfer, H. pers. comm.). The role of variability in plant abundance, plant quality and habitat characteristics still requires clarification (Mayhew, 1997). In the case of specialised species, which depend not only upon a high larval food quality, but also on enemy-free space and on certain habitat characteristics, rigid host plant selection is of a high adaptive value, since recognition and selection of suitable host plants can be performed in a short time window (Fletcher & Prokopy, 1991; Pyke, 1984). However if more acceptable plants are rare, less suitable plants may be considered for oviposition, too. However sawfly species retain eggs rather than oviposit in poor quality shoots of their host plants willow (Craig *et al.*, 1989). Adult life-span and abiotic conditions (Mayhew, 1997) or seasonal development (Obermaier & Zwölfer, 1999) may have additional effects on the acceptance of host plants by herbivorous insects.

In *U. cardui,* the females possess mainly mechano- and chemosensory receptors on their ovipositor (see **chapter 6**). They are able to measure the size of the apical meristem (see **chapter 7**) and presumably also the chemical environment of the corresponding tissue and perform first a yes-no-decision: if they decide to deposit eggs, they adjust the clutch size to the capacity of the axillary bud (see **chapter 7**). The morphology of the receptors present on the ovipositor may limit the host range of *U. cardui*. Parasitoids, galling and mining insect species, which all insert the eggs in plant or animal tissue usually analyse the quality of the host with extensive probing (Fitt, 1986; Vinson, 1985). The inner structure of the host (plant) is analysed with the receptors present on the ovipositor (Brown & Anderson, 1998; Le Ralec & Wajnberg, 1990; van Lenteren, 1972). Their morphology and processing of the incoming information determines the nature of the stimuli (Eisemann & Rice, 1985) that can be perceived: this may have implications on the host range. In the last ten years, a new synthesis of various theoretical areas of ecology resulted in the search for broad patterns in nature (Price *et al.*, 1995). It is assumed, that macro-evolutionary traits in a lineage set limits on the range of life history traits and behaviours, which can evolve (phylogenetic constraint hypothesis (Price, 1994)). One example for these limits is the evolution of ovipositor morphology, which limits the range of plant material that can be penetrated (Craig *et al.*, 1989; Price *et al.*, 1995).

A phytophagous insect depends on making an optimal decision, especially if it is searching for oviposition places, the suitability of which directly influences its own reproductive success (Fletcher & Prokopy, 1991). The host plant selection of herbivorous insects is on the one hand determined by the plant, especially in the case of gall formation, since the gall is induced by the insect, but formed by the plant (Béguinot, 2002; Weis *et al.*, 1988). On the other hand optimisation of larval performance exerts a high selection pressure on the searching, recognition and selection of the females (**chapters 2.1.2, 5 and 7;** Craig *et al.*, 1989; Fritz *et al.*, 2000; Price, 1994; Via, 1986). The latter effect should be also more pronounced in the case of plant galls, since the larvae are not able to leave their host plant (Ballabeni *et al.*, 2001). Nutritional quality of leaves, twigs or fruits can influence herbivore food choice, but in many cases chemical or structural defences of plant organs are the major determinants of palatability (Coley *et al.*, 1985; Diaz-Fleischer & Aluja, 2003). A better larval performance can be achieved when the larvae feed from plant tissue of a higher quality and if the larvae are protected from predators and parasitoids (Berdegue *et al.*, 1996; Biere *et al.*, 2002; Freese, 1995; Fritz *et al.*, 2003; Jeffries & Lawton, 1984). In contrast, the fitness of the plant increases if it can avoid being chosen as food source or oviposition place by an insect, since its own reproductive success will be diminished by insect feeding (Fay *et al.*, 1996; Feeny, 1975; Schoonhoven, 1990). Active use of defensive substances (Bernays & Graham, 1988; Feeny, 1990; Price, 1986) or storage of secondary plant metabolites like tannins or terpenoids (Feeny, 1970; Monaco *et al.*, 1982) are quite common. Nevertheless in evolutionary times phytophagous specialists evolved, species that are adapted to their host plant. They are no longer hindered by chemical or structural defences (Feeny, 1975). They may even be able to use them as indirect defences against their own enemies (Bernays & Graham, 1988; Coley, 1983; Price, 1986).

When a foraging female mainly suffers from mortality risks while travelling, patch residence time should be longest, it should be shortest when only within-patch mortality risks are important (Nonacs & Dill, 1991; Stoks & Johansson, 2000; Weisser *et al.*, 1994). All foraging strategies are rigorously shaped by natural selection and they should be considered in terms of degrees to which they maximize the net gain from feeding, and to which they minimize the risks to survival (Hassell & Southwood, 1978). A simple model of patch residence time and patch leaving (Rosenheim & Mangel, 1994) demonstrated that patch leaving is favoured by low travelling costs, high rates of self-superparasitized hosts, and high levels of parasitism in the currently occupied patch. In the case of *U. cardui* intraspecific parasitism is beneficial for the larvae, thus it will enhance the patch residence time of the females. The main predators of the adults are spiders (see **chapter 2.1.2**), thus the mortality in the patches is presumably higher than during travelling. Females of *U. cardui* are thus a subject to diverging influences on their residence time in a patch. In general it is necessary to include predators (for example Nomikou *et al.*, 2003), parasitoids (for example Micha *et al.*, 2000), bacteria (for example Raghu *et al.*, 2002) and fungi (for example Friedli, 2001) to provide a better understanding of all the complex interactions resulting from insect foraging strategies. It is favourable for the host plant if the herbivorous insect is diminished by parasitoids, but fungi growing on plants, which render the plant unattractive for the herbivorous insect, might have a negative effect on the plant itself.

Different foraging strategies are not only important for the organisms directly involved, but have also consequences for the population structure and density of other species on different trophic levels. In the *U. cardui* – *C. arvense* system only a small number of parasitoids are involved (see **chapter 2.1.2**). Three of the parasitoid species, *Eurytoma robusta* (Hymenoptera: Eurytomidae), *Pteromalus elevatus* (Hymenoptera: Pteromalidae) and *Torymus cyanimus* (Hymenoptera: Torymidae) attack also other tephritid species. Effects exerted on these species via the foraging strategy of *U. cardui* could additionally result in indirect effects on other host species in the habitat. The foraging strategy of one specialised insect can thus be a basic element of further interactions in the ecosystem, although generalists have a higher impact on further interactions.

The evolution of foraging behaviour should not be prevented by fixed genetic constraints (Pyke, 1984). This implies certain plasticity by genes, which act singly, thus no gene affects multiple traits of the behaviour and there is no linkage of several genes. There exist few experimental studies, which try to analyse links between fecundity, oviposition or foraging strategies and the underlying physiological and genetical processes (Berdegue *et al.*, 1996). This lack is mainly due to a lack of feasible methods. Recently several technologies have been invented, which allow at least a combination of physiology, behaviour and foraging to be investigated (Carlsson & Hansson, 2003; Groh *et al.*, 2002; Hansson, 2002; Larsson *et al.*, 2003; Stensmyr *et al.*, 2003; Stensmyr *et al.*, 2002; Strohm & Daniels, 2003; Zhang *et al.*, 1999). First it is necessary to analyse the host plant cues on which the herbivorous insect relies. Knowledge about them will be essential for development of experimental design to analyse the underlying processes and relationships to other aspects like fecundity, behaviour or morphology (Schoonhoven & Jermy, 1998). Studies like the one presented here may be a first step in the direction of a deeper comprehension of relevant process in ecosystems, insight into an important interaction between insects and plants. The analysis of the relevant senses males and females use for recognition and selection of territories, respectively oviposition places (see **chapter 5**) opens the possibility of performing tailor-made studies on the physiological processes.

It is generally assumed, that the high degree of specialisation in herbivorous insects can also be due to a higher efficiency of host plant recognition, if an insect concentrates on one certain species of host (Bernays, 1999). In evolutionary terms a specialised insect can afford a more finely tuned host (plant) recognition mechanism, than a generalist, since the host (plant) cues are more uniform (Janz & Nylin, 1997; Prokopy & Owens, 1978). It is thus assumed, that the insect is better able to judge the quality of the potential host. This implies a relatively rigid host plant template in specialised herbivorous insects (see **chapter 3**). A rigid host plant template leads to a higher efficiency of host plant recognition, selection and utilization (Fox & Morrow, 1981). It is also considered, that rigid host plant templates are favoured by neural constraints (Bernays, 1999; Janz & Nylin, 1997). But in evolutionary terms such a rigid template may also prevent the radiation of a certain species. It is possible, that the insect is not only prevented from the use of other hosts, but that it may be unable to follow even evolutionary changes of the host species (Jaenike, 1990; Larsson & Ekbom, 1995).

Another aspect makes the foraging strategy of *U. cardui* interesting in respect to the evolutionary background: the influence of male territory selection on female host plant choice (see **chapter 5.3.5**). The marking substance of the males guides the females to suitable, enemy-free plants, which additionally host a mating partner. This system is unique, since each male selects the oviposition place for the offspring of its predecessor (see **chapter 2.1.2**). Though the quality of its decision affects the male’s mating success, the fitness of its offspring is determined by the choice of its successor.

As outlined above several aspects of the present study provide interesting new aspects for future work. A study of the host finding mechanisms of natural enemies of *U. cardui* in experimental studies could provide a deeper insight into the influence of upper trophic levels on the host plant selection of the specialised gall fly *U. cardui.* A second possibility to carry on the present study would be the incorporation of the detected aspects of the foraging strategy of *U. cardui* (see **chapter 8**) into general models of foraging strategies. It will be interesting to see how the long-term effect of *U. cardui* on its host plant *C. arvense* will develop in Canada. This may shed new light on the foraging process, too.

Especially concerning the evolutionary point of view, an extremely important topic in future research should be the analysis of the connection between the aspects of the foraging strategy already analysed and the underlying physiological processes. Studies on neuroethology of oviposition decisions or behaviour of insects in general are rare (Carlsson & Hansson, 2003; Larsson *et al.*, 2003; Tousson & Hustert, 2000). Only recently Stensmyr *et al.* (2003) were able to show *how* olfactory receptor neurons can inform an insect on the food in its vicinity. Thereby the olfactory receptor neurons match pheromone olfactory neurons with respect to selectivity and sensitivity. Hopefully these new physiological techniques will be also applicable to such small species as *U. cardui* in the future, to provide the necessary tools to gain deeper insight into the foraging strategy of a specialised insect. This could add information to various areas of biological sciences (like for example evolution, food web theory, behavioural ecology, physiology and chemical ecology) and lead scientists to a better understanding of the key interactions between plants and insects in ecosystems.

The present study on the complex foraging process for host plants and oviposition sites on these was the first study to detect an influence of the foraging of males for territories and is thus the first to include male behaviour in respect to oviposition. Males and females of *U. cardui* seem to use different cues of their host plant. Since each male pre-selects the oviposition site of its predecessor the evolution of this highly specialized system is extremely interesting.

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10 Summary

Nearly all herbivorous insects in terrestrial ecosystems depend on plants for their survival and reproduction. Additionally they dominate all terrestrial ecosystems due to species and individual abundance. Interactions between these two groups are thus of a high significance for the analysis and the understanding of the complex interactions in terrestrial ecosystems. Especially foraging for host plants by herbivorous insects is of a central importance.

In the present thesis the foraging strategy of the specialised gall fly *Urophora cardui* on the creeping thistle, *Cirsium arvense,* was investigated as a model system for the foraging process of a specialised herbivorous insect.

Males and females of *U. cardui* use the larval host plant as their rendezvous place. The males establish territories on the plant, which they defend against conspecific males. The females lay eggs into axillary buds, in order to initiate gall development.

- Male and female body size, measured as weight at eclosure or capsule width, was not correlated with male respectively female longevity. Generally males lived shorter than females. Capsule width of males and females was not significantly different, while weight at eclosure and fresh weight at death was. Females weighed more, which may be due to their higher need of energy during adult life. Both sexes lost body weight during life, at their death there was still a significant difference between the sexes, but it was less prominent than at eclosion.
- All behaviours, which are performed by males and females on their host plant were defined, recorded and analysed. The females spent most of the time they were on a plant on resting, probing axillary buds, running on the plant and grooming. The males spent most of the time they are on a particular plant on copulation and patrolling their territory. The behaviour of both sexes was highly variable between individuals. Concerning the movement pattern on an already chosen host plant, the females concentrate on the upper parts of the host plant. They were mainly occupied with an extensive probing of various axillary buds, which occurred with a higher probability in a suitable developmental stage at the top of the plant. In contrast the males patrolled the whole plant, although only the upper leaves became marked. If they encountered another male on their territorial plant threatening and fighting were inevitable. Fights lasted several hours interrupted by threatening periods. Mating of males and females usually followed oviposition.
- Neither females nor males accepted modified host plants or models of thistles. Their behaviour on modified thistles was reduced mainly to running around the plant and grooming, if they stayed long enough to enable observation. These results indicate a rigid host plant template using the input of several senses, thus the flies always recognise models and modified plants as a non-host plant.
- Both sexes were able to discriminate host plants and non-host plants from a distance of 0.8-2m. They decided for a particular plant already from this distance and flew immediately to the selected one, before they really entered the host plant stand.

- The time male and female flies needed until they selected one of the host plants in a particular host plant stand depended on the number of non-host plants, host plants and the number of suitable hosts. The decision-time became shorter, if there were not too many suitable host plants. This may be due to decreasing sampling time of the host plants present.
- *U. cardui* females did not prefer plants of a certain height. In contrast the branching level, which indicates the number of axillary buds, and the number of flower buds played a significant role during foraging for host plants. Plants with an intermediate branching level were preferred, while those with many flower buds, indicating a higher age, were avoided.
- The males of *U. cardui* were able to select their territorial plant on olfactory cues or on visual cues likewise. In contrast, the females were not able to recognise their host plant on olfactorial cues alone. But, if male-marked and unmarked host plants were available they significantly preferred the marked thistles. The differentiation between marked and unmarked *C. arvense* were made according to olfactorial cues, since the plants did not differ in their height, nor in their branching level, nor in the number of flower buds. Thus, female selection of larval host plants depended on male choice for territories. This result is remarkable, especially in evolutionary terms, since the males select the larval host for the offspring of their predecessor. Interestingly the males preferred plants marked by conspecifics also.
- On the tip of the females' ovipositor there is a receptor field with several morphological different receptor types. Mainly they seem to have mechanoreceptive as well as chemosensory functions. According to studies on other insect species they may also be able to analyse the water-content of the tissue. These receptors enable the females to measure and analyse the inner structure of the plant tissue at the axillary bud.
- The width of the apical meristem of the axillary buds was proven to influence female choice of the plant organ, where eggs become deposited. Axillary buds with an apical meristem-diameter above 0.62mm had a higher probability to became chosen by the *U. cardui* females. The clutch size was adjusted to the diameter of the apical meristem, indicating, that the females were able to estimate the quality of the respective axillary bud, which influences larval performance.

These results were summarized in a general model of the foraging strategy of *U. cardui*, as an example for other specialised herbivorous insects.

11 Zusammenfassung (German Summary)

Herbivore Insekten sind elementar von Pflanzen abhängig, nicht nur in ihrem Bestreben zu leben sondern auch in der Fortpflanzung. Als arten- und individuenreichste Gruppen in terrestrischen Ökosystemen stellen Pflanzen und Insekten die wichtigsten Gruppen dar. Interaktionen zwischen diesen beiden Gruppen sind deshalb von enormer Bedeutung für das Verständnis der komplexen Vorgänge innerhalb eines solchen Systems. Insbesondere der Wirtswahl herbivorer Insekten kommt hier eine zentrale Rolle zu.

In der vorliegenden Arbeit wurde die „foraging-strategy" der spezialisierten Gallfliege *Urophora cardui* an der Ackerkratzdistel, *Cirsium arvense,* als ein Modellsystem für die Wirtswahl eines spezialisierten herbivoren Insektes, untersucht.

Die Männchen und Weibchen benutzen die Wirtspflanze der Larven als „Rendezvous"-Platz, wobei die Männchen an dieser Pflanze Territorien errichten, die sie gegen andere Männchen verteidigen. Die Weibchen legen Eier in die Knospen junger Seitentriebe. Dadurch wird die Bildung einer Pflanzengalle initiiert.

- Die Größe von Männchen und Weibchen, gemessen als Schlupfgewicht oder als Kopfkapselbreite, korrelierte nicht mit der Lebensdauer der Individuen. Männchen lebten generell kürzer als die Weibchen. Die Kopfkapselbreite der beiden Geschlechter unterschied sich nicht signifikant, während sowohl das Schlupfgewicht, als auch das Todesgewicht signifikant verschieden waren. Die Weibchen waren schwerer als die Männchen, was wahrscheinlich mit einem höheren Energiebedarf zusammenhängt. Individuen beider Geschlechter verloren im Laufe ihres Lebens an Gewicht, wobei die Differenz zwischen Männchen und Weibchen zum Todeszeitpunkt geringer als zum Schlupfzeitpunkt war.
- Alle Verhaltensweisen, die von Männchen und Weibchen an ihrer Wirtspflanze ausgeübt werden, wurden definiert, aufgenommen und analysiert. Die Weibchen verbrachten die meiste Zeit, die sie auf einer bestimmten Pflanze waren, damit zu ruhen, die Seitenknospen zu testen, die Pflanze zu belaufen, oder sich zu putzen. Die Männchen dagegen verbrachten die meiste Zeit, die sie sich auf ihrem Territorium befanden, damit zu kopulieren oder ihr Territorium zu patrouillieren. Das Verhalten beider Geschlechter war individuell sehr variabel. Was das Bewegungsmuster an sich betrifft, so befanden sich die Weibchen hauptsächlich am oberen Teil der Wirtspflanze, wo die Wahrscheinlichkeit, geeignete Seitenknospen zu finden, höher ist. Im Gegenteil dazu beliefen die Männchen die gesamte Wirtspflanze, wobei sie aber überwiegend die oberen Blätter mit dem Sekret ihrer Rektaldrüse markierten. Sobald sie auf ein anderes Männchen derselben Art trafen waren gegenseitiges Drohen und ein folgender Kampf unausweichlich. Derartige Kämpfe konnten bis zu mehrere Stunden in Anspruch nehmen. Die Paarung von Männchen und Weibchen erfolgte normalerweise nach der Eiablage durch die Weibchen.
- Weder die Männchen noch die Weibchen akzeptierten modifizierte Wirtspflanzen oder Distelmodelle. Ihr Verhalten auf modifizierten Wirtspflanzen war hauptsächlich auf das Belaufen der Pflanze sowie das Putzen reduziert, sofern

sie überhaupt lange genug auf der Pflanze blieben, um eine Beobachtung zu ermöglichen. Die Ergebnisse deuten auf ein sehr starres Wirtspflanzen - Suchbild sowie die Nutzung verschiedener sensorischer Modalitäten hin. Dadurch sind die Fliegen immer in der Lage, eine veränderte Pflanze oder ein Distelmodell als eine Nicht - Wirtspflanze zu erkennen.

- Beide Geschlechter konnten eine Wirtspflanze aus einer Entfernung von 0.8-2m erkennen. Sie entschieden sich für eine bestimmte Pflanze schon aus dieser Distanz, noch bevor sie einen Standort mit mehreren Wirtspflanzen richtig erreicht hatten.
- Die Zeit, die die Männchen und die Weibchen jeweils benötigten, bevor sie sich für eine bestimmte Pflanze an einem Standort entschieden, hing von der Anzahl der Nicht – Wirtspflanzen, der Zahl der Wirtspflanzen und der Zahl der geeigneten Wirtspflanzen ab. Die Entscheidungszeit wurde kürzer, wenn weniger geeignete Wirtspflanzen vorhanden waren. Dies ist wahrscheinlich auch darauf zurück zu führen, dass die Individuen weniger lange brauchten, um die vorhandenen Pflanzen zu evaluieren.
- Weibchen von *U. cardui* bevorzugten keine bestimmte Höhe ihrer Wirtspflanze. Der Verzweigungsgrad, der die Anzahl der Seitenknospen anzeigt, sowie die Anzahl von Blütenknospen spielten jedoch eine wichtige Rolle bei der Auswahl. Pflanzen mit einem mittleren Verzweigungsgrad wurden signifikant bevorzugt, während solche mit vielen Blütenknospen, die ein höheres Alter der Pflanze anzeigen, abgelehnt wurden.
- Männchen von *U. cardui* waren in der Lage, ihr Territorium entweder anhand von olfaktorischen oder anhand von visuellen Signalen auszuwählen. Im Gegensatz dazu waren die Weibchen nicht in der Lage ihre Wirtspflanze anhand des olfaktorischen Profils zu erkennen. Aber wenn sowohl von Männchen markierte Pflanzen wie auch unmarkierte zur Wahl gestellt wurden, so bevorzugten die Weibchen signifikant die markierten Pflanzen. Die Differenzierung von markierten und unmarkierten Pflanzen kann dabei nur aufgrund der olfaktorischen Signale erfolgt sein, da die jeweils zur Wahl stehenden Pflanzen weder in der Höhe, noch im Verzweigungsgrad, noch in der Anzahl der Blütenknospen unterschiedlich waren. Die Wahl der Weibchen für eine Wirtspflanze hing somit von der Wahl der Männchen für ein Territorium ab. Dieses Ergebnis ist besonders für die Evolution bedeutsam, denn die Männchen wählen dadurch die Wirtspflanze für den Nachwuchs ihres Vorgängers aus. Interessanterweise bevorzugten die Männchen ebenfalls Pflanzen, die von Artgenossen markiert worden sind.
- An der Ovipositorspitze besitzen die Weibchen von *U. cardui* ein rezeptives Feld mit mehreren, morphologisch unterschiedlichen Rezeptoren. Hauptsächlich scheinen sie sowohl mechanorezeptive, sowie chemorezeptive Funktionen zu haben. Nach ähnlichen Studien in anderen Insektenarten ist es auch denkbar, dass die Rezeptoren in der Lage sind den Wassergehalt des Gewebes zu bestimmen. Die Weibchen sind in der Lage, mit Hilfe des rezeptiven Feldes die innere Struktur der Seitenknospe zu analysieren.
- Es konnte nachgewiesen werden, dass der Durchmesser des Wachstumspunktes der jeweiligen Seitenknospe einen signifikanten Einfluss auf die Wahl des

> Eiablageortes der Weibchen hatte. Seitenknospen mit einem Durchmesser des Wachstumspunktes von über 0.62mm haben eine höhere Wahrscheinlichkeit, dass sie für die Eiablage ausgewählt werden. Die Gelegegröße wurde von den Weibchen dem Durchmesser des Wachstumspunktes angepasst. Dies deutet darauf hin, dass die Weibchen in der Lage sind, die Qualität der betreffenden Seitenknospe, die sich auf die Überlebenswahrscheinlichkeit der Larven auswirkt, abzuschätzen.

Diese Ergebnisse wurden in einem allgemeinen Modell der „foraging-strategy" von *U. cardui,* als ein Beispiel für die Wirtspflanzenwahl eines spezialisierten herbivoren Insektes, zusammen gefasst.

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13 Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig verfasst habe. Dabei kamen nur die angegebenen Quellen und Hilfsmittel zur Anwendung.

Weiterhin erkläre ich, dass ich weder diese noch eine gleichartige Doktorarbeit an einer anderen Hochschule eingereicht, noch anderweitig mit oder ohne Erfolgung versucht habe mich der Doktorprüfung zu unterziehen.

Wiltrud Daniels, Bayreuth den 31.05.2004